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DIFFUSE ANGIECTASIS OF THE CEREBRAL MENINGES OF THE NEWBORN INFANT

Report of Three Cases

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A LOCALIZED increase in the number, the size or the tortuosity of the vessels of the meninges covering the cerebrum or the cerebellum has been described by numerous observers. The increases have been variously situated, and in many instances the vessels have extended into the brain beneath the areas of meningeal involvement. They have often been associated with abnormalities of blood vessels in other locations, especially the face and the retina.

An extensive search of the literature has failed to reveal descriptions of vascular abnormalities generalized over the surface of the brain in any age group similar to those to be described in the present report. In a few previously reported cases the entire meningeal area exposed at operation was abnormal, and it may be possible that the lesion involved more of the meninges than was apparent to the surgeon. Against this possibility is the fact that in most instances definite localizing symptoms were present. Such a case was reported by Bailey,[1] and his description of the localized tumor of his patient applies almost exactly to the apeparance of the more widespread lesions found at postmortem examination in the present cases. Bailey stated that "all over the exposed cortex the vessels were greatly dilated. In the temporal and parietal regions were tangled masses of small vessels and deep in the temporal region were more tortuous vessels which could not be exposed."

As far as I have been able to determine, tumors arising from meningeal vessels or malformations of meningeal vessels which might be interpreted as angioma or other types of vascular tumor have never been described as observed in fetuses or young infants.

The present report concerns 3 newborn infants whose meningeal vessels showed remarkable changes of gross appearance. In each instance there was also an abnormality of the heart.

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1. Bailey, P.: Intracranial Tumors, Springfield, Ill., Charles C Thomas, Publisher, 1933.

The vessels of the meninges of 2 infants were almost identical in appearance (fig. 1). Over the entire surface of the cerebrum and the cerebellum these vessels were of fairly uniform size and exhibited a great increase in number and tortuosity. They formed a scroll-like pattern, and their many convolutions produced a generalized tangled mass over the exterior of the brain. They had no connection with the vessels of the brain or those of the dura mater, and all were confined to the leptomeninges. The membrane holding the vessels together was fragile and easily torn. When this membrane and its associated blood vessels were removed, the underlying surface of the brain was smooth, white and normal in appearance. The histologic structure of the brain was normal.

These meningeal blood vessels had the structure of capillaries and terminal veins. Many vessels were composed of a single layer of endothelial cells, while others were made up of endothelium surrounded by a few muscle cells. In the third infant the appearance of the meningeal vessels was somewhat different, although here, too, a scroll-like tortuosity was the principal abnormality (figs. 4 and 5). The increase of the total number of vessels was somewhat less striking than in the other infants, and the proximal portions were of considerably greater caliber than were the distal branches. Greater irregularity of the size of the venous channels was thus produced, and nowhere was there such great concentration of vessels as was present throughout the meninges of the other infants.

Each of the 3 infants had an associated abnormality of the heart. In 2 it consisted of generalized hypertrophy. The size of the chambers was abnormally great, and the muscle was increased in thickness, but the valves and the great vessels leading from the heart were normal (figs. 2 and 3). No glycogen could be demonstrated in the muscle cells. The third infant had only slight enlargement of the heart, but the left innominate vein entered the left atrium instead of joining the right innominate vein to form the superior vena cava (fig. 6). This infant also had an abnormality of the liver consisting of the presence of numerous superficial veins, lying immediately beneath the capsule, a diffuse dilatation of the veins in the center of the lobules and a moderate increase in periportal connective tissue.

It seems more than coincidence that this abnormality of cerebral vessels has been observed on three occasions in infants who also had abnormalities of cardiac development. If the changes in the meningeal vessels are related to cardiac hypertrophy, it is interesting to speculate on cause and effect. Could it be that the heart hypertrophied in order to supply the increased vascular bed in the meninges, or could the hypertrophy of the heart have caused a sufficient increase in cardiac

output and pressure to be responsible for hyperdevelopment of the meningeal vessels? In either instance one would expect the arterial system to be as greatly or more greatly involved than the venous, and in all 3 infants the arteries in the body appeared normal.

It is also interesting to speculate on the symptoms that would have been produced by this great increase in meningeal vessels had the infants survived. The fact that it is not a condition recognized in later life suggests that it may be incompatible with continued existence and that all those affected die in early infancy.

REPORT OF CASES

CASE 1.—The mother was a 23 year old primipara whose pregnancy was normal and who was delivered at term by low forceps after twenty-four hours of labor. The fetal heart tones disappeared during labor, and the infant was dead at birth. A twin weighing 935 Gm. was born ten minutes after the first infant. Maceration was extreme, and no abnormalities could be made out.

The first twin was a boy weighing 3,832 Gm. and measuring 56.5 cm. in total length. Moderate generalized edema which was most severe in the region of the ears and the eyelids was present. The tongue protruded slightly from the open mouth. No fluid was present in the chest, but about 50 cc. was found in the abdominal cavity.

The most notable changes were in the meninges, the heart and the thyroid gland. The meninges covering the entire brain contained vessels which were greatly increased in length and number. The surface was so covered with the innumerable loops and coils of the venous channels that the underlying brain was almost invisible (fig. 1). The vessels were of approximately uniform caliber, were distended with blood and in no place penetrated the brain. In appearance they were similar to capillaries and thin-walled veins; many of the vessels were made up of a single layer of cells. No vessels having the structure of arteries were found in the involved areas.

The heart was remarkably enlarged and weighed 68 Gm. (fig. 2). All its parts were increased in size as a result of hypertrophy of the cardiac muscle and enlargement of the chambers. The valves, the great vessels and the septums were normal. The histologic structure of the cardiac muscle was normal, and glycogen could not be demonstrated in the cells.

The thyroid gland was remarkably increased in size and weighed 6.5 Gm. The vessels were distended with blood and were conspicuous, although no actual increase in number appeared to exist. The acini contained no colloid, and the lining cells were partially desquamated. In spite of the increased size of the gland, no changes which were clearly pathologic could be made out.

The lungs were hypoplastic as a result of being directly compressed by the enlarged heart, and their combined weight was 20 Gm.

The remaining organs were within normal limits in size and histologic structure. The thymus was somewhat smaller than usual, and the liver contained larger and more numerous areas of erythropoiesis than are ordinarily present; similar findings, however, may occasionally be present in otherwise normal infants. The majority of the blood vessels throughout the body were distended with blood.

CASE 2.—The mother was a 40 year old Negro woman who had had seven normal pregnancies. Her antepartum course was uneventful. She went into labor spontaneously and was delivered by breech extraction after a total labor of nine

hours. Membranes had ruptured about twenty-four hours before the onset of labor. The infant breathed immediately after birth but subsequently became dyspneic and cyanotic. These symptoms became progressively worse, and death occurred twenty-five hours after birth.

The infant was a girl, weighing 5,810 Gm. and measuring 60 cm. in length. No external abnormalities were visible, and much of the increase in weight was due to an excessive deposit of subcutaneous adipose tissue.

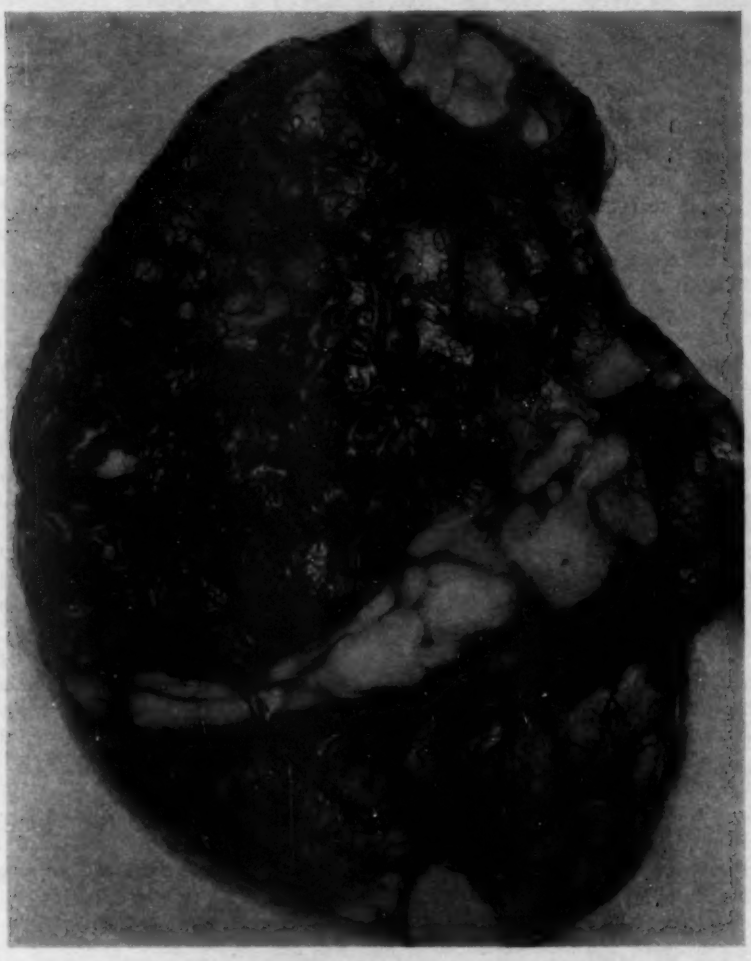

Fig. 1 (case 1).—Surface of the brain, showing angiectatic capillaries and veins in the leptomeninges. The white areas are artefacts caused by tearing of the arachnoid membrane during removal of the brain.

The meninges and the heart were remarkably similar to those described in case 1. The vessels of the meninges were excessive in number and length and formed a tangled mass which was generalized over the entire surface of the brain. They did not penetrate the surface of the brain but remained confined to the meninges. The appearance was similar to that reproduced in figure 1.

Fig. 2 (case 1).—Opened thoracic and abdominal cavities showing the extremely hypertrophied heart almost completely filling the thoracic space.

Fig. 3 (case 2).—Thoracic cavity showing extreme cardiac hypertrophy.

The heart also was similar to that of case 1 except that the apex was more pointed and the atriums were not proportionately as greatly increased (fig. 3). The ventricular and atrial walls were thickened, and the chambers were enlarged. The valves and the great vessels were normal. The weight was 71 Gm.

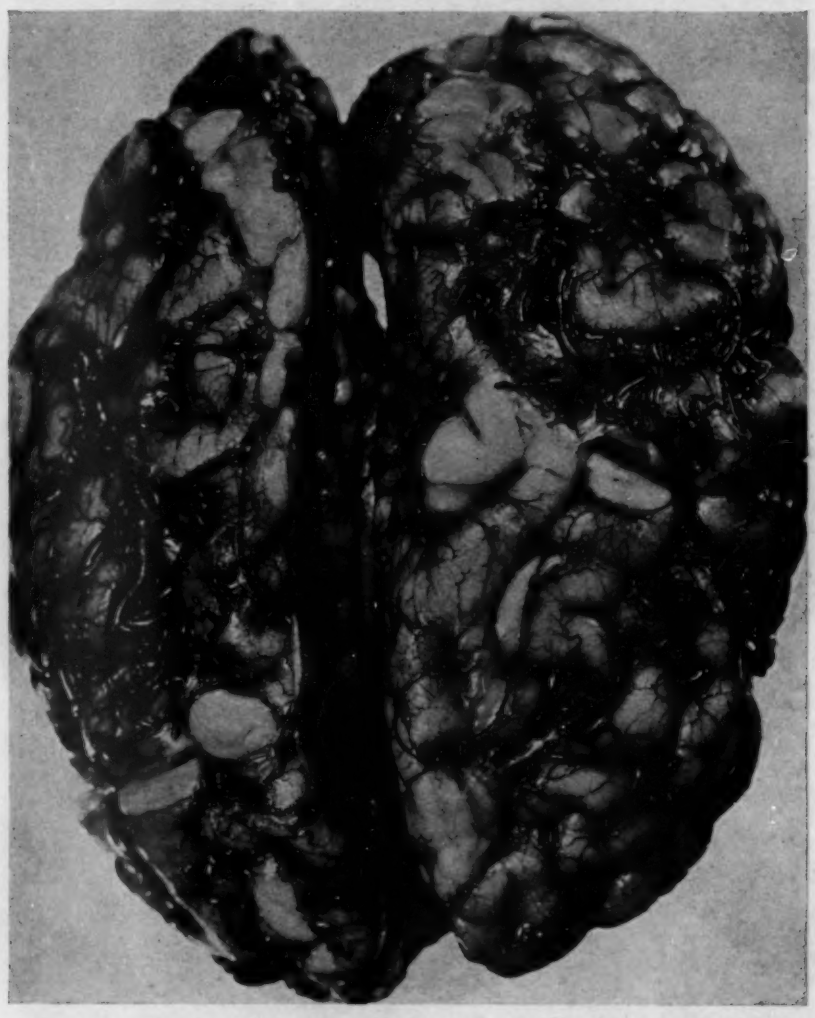

Fig. 4 (case 3).—Superior surfaces of the cerebral hemispheres, showing dilated angiectatic veins and capillaries in leptomeninges.

The lungs appeared to be normally developed and were increased in weight as a result of pneumonia. The pneumonic process may have been caused by the aspiration of amniotic fluid following premature antepartum rupture of the membranes. The lungs weighed 120 Gm.

The thyroid gland was slightly enlarged and weighed 3.5 Gm. The vessels were greatly distended, no colloid was present and the cells lining the acini were partially desquamated.

Mild erythropoiesis was present in the liver. The remaining organs showed nothing of note except extreme congestion.

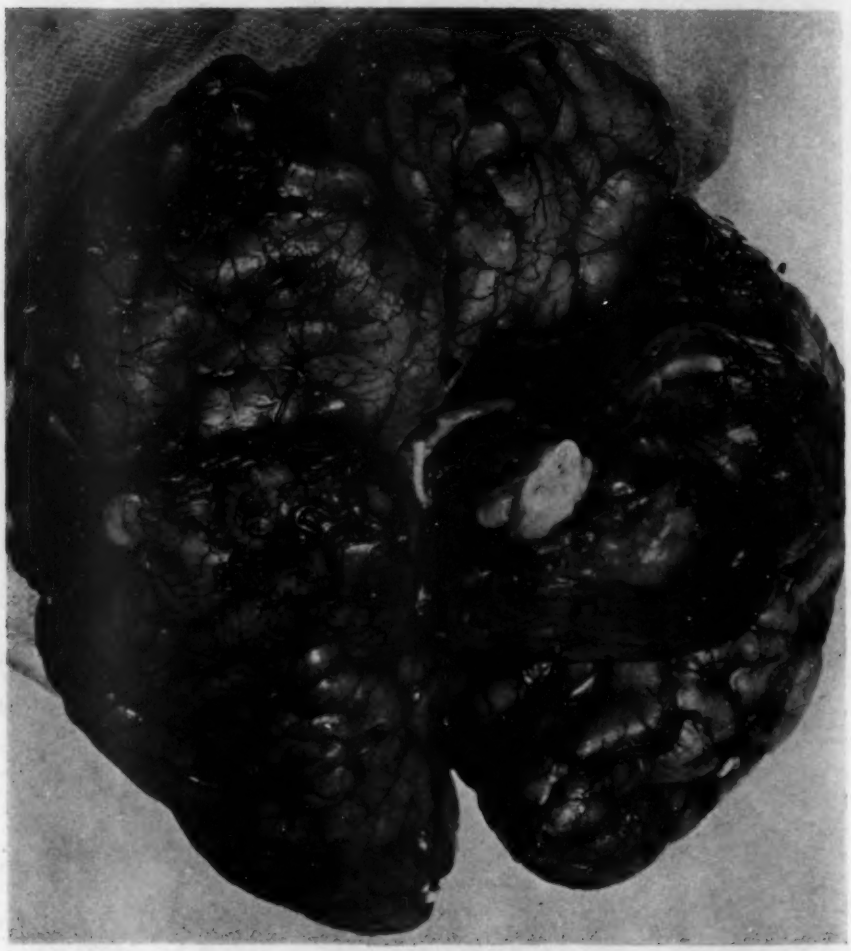

Fig. 5 (case 3).—Inferior surfaces of the cerebral hemispheres and the cerebellum, showing tortuous, scroll-like vessels similar to those on the superior surfaces.

Case 3.—The mother was a 25 year old woman who had had one normal pregnancy. She had symptoms of mild toxemia and was admitted to the hospital four times during pregnancy because of abdominal pains which were thought to threaten early termination of pregnancy. She went into labor spontaneously at thirty-eight weeks and was delivered by low forceps after nineteen hours of labor.

Fig. 6 (case 3).—Opened body cavities showing (1) the left superior vena cava entering the left atrium and (2) dilated, abnormally placed veins on the surface of the liver.

The infant was cyanotic at birth, and respiration was established with difficulty. The cyanosis continued, respiration was of poor quality and death occurred after thirty-two hours.

The infant was a boy weighing 2,900 Gm. and measuring 48 cm. in length. As in the other 2 infants, the meninges and the heart were the site of the principal lesions. The vessels of the meninges, however, were somewhat different; the principal vessels were increased in caliber, and the smaller vessels, although tortuous and circinoid, were involved to a somewhat lesser degree than those of the other infants. (Compare figures 4 and 5 with figure 1.) The brain substance was normal.

The heart was stated to have been enlarged, but it was not weighed, and the increase in size does not seem to have been appreciable. The left innominate vein, instead of uniting with the right innominate vein to form the superior vena cava, entered the lateral wall of the left atrium anterior to the pulmonary veins. The membrane covering the foramen ovale was unusually thick and was herniated into the right atrium. The heart was otherwise normal.

The liver of this infant was also abnormal and was covered superficially with prominent, dilated veins. The central veins within the liver were dilated and unusually prominent. Connective tissue was present in excessive amounts in the periportal areas and in lesser amounts in irregular areas between the lobules.

SUMMARY

Three infants suffered an abnormality of development in the meningeal vessels over the entire surface of the brain. The vessels were similar to those found in localized lesions in older persons which have been described under the name of meningeal angioma. The infants described in the present report had a generalized involvement of all meningeal vessels producing a diffuse angiectasis in the cranial meninges. A cardiac abnormality was also present in each infant.

SPECIFIC GRAVITY OF THE BLOOD CORPUSCLE

Its Possible Significance in Atherosclerosis

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IN A previous communication I [1] suggested that the lipophage, because of its high content of fatty esters, would have a lower specific gravity than the other cells of the blood and thus be the first to enter the peripheral zone of the blood stream when the velocity diminished, and so be in a situation to enter the intima of the elastic arteries, causing the primary lesion in atheroma. In common with the authors of all current textbooks of pathology I accepted rather uncritically the hypothesis that cells of the blood of low specific gravity did leave the axial stream first because of their specific gravity. Schklarewsky [2] first suggested this, but no one except Hamilton [3] has apparently ever considered why it should be so and Hamilton's 63 year old explanation is certainly wrong, as it is based on the assumption that the erythrocytes have a specific gravity approximating that of the plasma and that the white corpuscles are lighter than the plasma. In fact, Fahreus [4] and his pupil Vejlens [5] have demonstrated clearly that the position of a cell in the blood stream may depend on its size, the larger cells being carried more centrally in the vessel than the smaller ones. From this demonstration they have decided that the specific gravity can be of no importance whatsoever, that Schklarewsky, being a Russian working in Germany and not understanding German properly, did not really mean that specific gravity was important when he said so, and that the leukocytes in his experiments left the axial stream first because in the frog leukocytes are smaller than erythrocytes. In man, of course, leukocytes are larger than erythrocytes, but when the current is slow enough the erythrocytes agglutinate and the clumps are then larger than the individual leukocytes.

While agreeing that size is one factor influencing the place of a corpuscle in the blood stream I feel that, for reasons to be discussed,

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From the Public Health Offices.

1. Gordon, I.: Arch. Path. **44**:247, 1947.
2. Schklarewsky, A.: Arch. f. d. ges. Physiol. **1**:602 and 657, 1868.
3. Hamilton, D. J.: J. Physiol. **5**:66, 1884-1885.
4. Fahreus, R.: Physiol. Rev. **9**:241, 1929.
5. Vejlens, G.: Acta path. et microbiol. Scandinav., 1938, supp. 33.

it is not the only factor or even the main one. If the corpuscles in a vessel were confined to a cylinder two-thirds the diameter of the vessel they would form a solid mass. This does not happen; so some force or forces must keep the corpuscles apart as well as draw them to the center. The story about Schklarewsky is rather weak, for Thoma,[6] a German, worked with Schklarewsky in the laboratory of Helmholtz, and Thoma, in his textbook, has been emphatic about the influence of specific gravity, and he could not have had trouble with the language. I feel that all parties to the discussion have ignored a fundamental fact, that the place of a corpuscle in the blood stream must also depend on its contacts with the erythrocytes.

In the male 47 per cent, in the female 42 per cent, of the volume of the blood consists of red blood corpuscles; thus the space between erythrocytes is not much greater than the volume of the erythrocyte itself. Furthermore, in the peripheral regions, especially, these corpuscles are traveling at different velocities. Except for the lightest gases, fluids in tubes flow either in laminar or in turbulent fashion. In the smaller vessels the flow is undoubtedly laminar, as this has been demonstrated cinematographically by Knisley and associates.[7] The type of flow in the aorta and the larger elastic arteries has not been determined, but even if it is turbulent, there is a steep gradient of velocities near the periphery. The type of distribution of velocities in turbulent flow may be illustrated as in figure 1 *A;* and laminar flow in a fluid containing a large number of corpuscles is, according to Vejlens,[5] somewhat similar, the paraboloid shape of laminar flow in water being blunted as in figure 1 *B*. In either case there is a thin boundary layer where the velocity steeply falls to zero; inside this layer in laminar flow the blood moves regularly in laminas, the nearer the center the more swiftly; in turbulent flow it proceeds in eddies. In laminar flow there is probably little bumping of one red corpuscle with another, for the erythrocytes (Knisley and associates[7]) also flow in laminas, each one corpuscle thick. A corpuscle larger than an erythrocyte, however, would be subject to many collisions.

EFFECT OF CONDITIONS IN THE SMALLEST VESSELS

According to Fahreus[4] and Vejlens,[5] in what they termed the "para-capillary" vessels the leukocytes, being larger than the erythrocytes, flow nearer the center. However, when the velocity is reduced, or in certain other circumstances, the red cells clump together, and these aggregates, being larger than the leukocytes, flow in the center. The

6. Thoma, R.: Text Book of General Pathology and Pathological Anatomy, London, Adam & Charles Black, 1896.

7. Knisley, M. H.; Block, E. H.; Eliot, T. S., and Warner, L.: Science **106**: 431, 1947.

authors then gave this changing of position as sufficient reason why the leukocytes begin to appear in the hitherto cell-free peripheral zone. It is difficult to see their reasoning, for the axial zone is still the same size and contains no more cells; in fact, Fahreus stated that the blood corpuscles are diluted in these paracapillary vessels, and thus there is even more room for them in the axial stream. It is here that another mechanism must be considered. The erythrocyte clumps (or sludges as Knisley called them) traveling more centrally also thus travel more quickly. The red cells, being nearly half the volume of the blood, frequently overtake the leukocyte on its inner side and collide with it. The forces concerned are, in respect of the bumping corpuscle, its kinetic energy and, in respect of the bumped corpuscle, its inertia. Kinetic energy is expressed as $\frac{1}{2} m V^2$ (m — mass and V — velocity—in this case the difference between the velocity of the leukocyte and that of the agglutinate). The inertia of the bumped

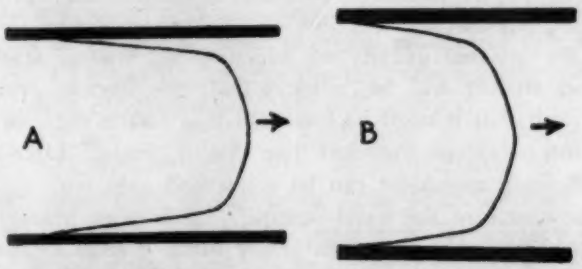

Fig. 1.—*A*, diagrammatic representation of the distribution of the differing velocities in a blood vessel with turbulent flow; *B*, that in a vessel with laminar flow.

corpuscle also depends on its mass. Now mass is the product of volume and specific gravity; the volume of the red cell aggregate is greater than the volume of the leukocyte; its specific gravity is greater (the specific gravity of the red blood cell is 1.094; that of the leukocyte, 1.061, according to Vejlens [6]). The leukocyte that is not on the edge of the axial stream would in its turn bump the more slowly flowing erythrocyte masses on its outer side, but would have a smaller effect in dislodging them, as its mass is so much less, and, on receiving impacts on its inner side when space is available between red cell clumps, would be bounced out. The reaction of the leukocyte would, however, be the resultant of two forces; it would be bounced out only when the colliding forces are greater than the force tending to draw it toward the center. Thus the leukocyte, because of its smaller mass, would tend to be bounced out of the axial into the peripheral zone. In this restricted sense, then, specific gravity is of importance, as it is a factor in the determination of mass.

EFFECT OF CONDITIONS IN THE ELASTIC ARTERIES

Although Knisley and associates [7] have found sludged blood in hypertension and arteriosclerotic heart disease, it would be wrong to assume that sludged blood is always present in the aorta when that vessel is atheromatous. If the blood is sludged, the lipophages would be bounced out by the heavier erthrocyte masses when the indrawing forces are lessened, as described earlier.

That the lipophage has a lower specific gravity than the polymorphonuclear leukocyte or the lymphocyte is almost certain. The specific gravity of the rabbit polymorphonuclear leukocyte is about 1.061; that of cholesterol is 1.046, and that of its esters would be even less. I have been unable to obtain the specific gravity of pure cholesterol esters, but there is no reason to assume that the lipophage is full of pure esters only; it probably contains other fat-soluble substances as well, and in fact, if one is to judge by the fat content of the atheromatous intima (Page [8]), it probably contains a considerable proportion of neutral fat. An impure mixture of cholesterol esters is anhydrous wool fat, the specific gravity of which is as low as 0.0940.[9] The only correct answer will be, when found, the specific gravity of the lipophage itself, but it must be less than that of the polymorphonuclear leukocyte and is perhaps even less than that of plasma (1.028).

The following argument can be concerned only with lipophages in the boundary zone of the axial stream. Leukocytes nearer the center will obviously be out of the aorta before much change in their position can be effected, and since the stream is probably turbulent, the argument will not apply. The size of the lipophage from which the mass is deduced is of great importance, for in the case now considered the erythrocytes flow individually, i. e., not in sludges, and thus have a much reduced mass. Anitschkow [10] stated that the lipophages may vary in size from that of a lymphocyte to that of a large monocyte, and Leary [11] has pointed out that they are small in the lung capillaries and tend to become larger in the tissues. At times they are big, and Beard and Rous [12] have described Kupffer cells, from which they are derived, growing to the fantastic size of 540 microns. This increase in size increases the mass, and so also the inertia, but with increase in size it can be shown that there is a corresponding increase of packets of kinetic energy striking the cell. Those lipophages of the size of polymorpho-

8. Page, I. H.: Biol. Symposia **11**:43, 1945.

9. Codex Medicamentarius Gallicus: Pharmacopée Française, ed. 6, Rennes, Imprimeries Oberthur, 1937, vol. 2.

10. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

11. Leary, T.: Arch. Path. **32**:507, 1941.

12. Beard, J. W., and Rous, P.: J. Exper. Med. **59**:593, 1931.

nuclear leukocytes or less would have less inertia than these cells and so be more easily displaced out. It is difficult to see how any leukocytes can be drawn into the center as Fahreus [4] described, as they are continually being overtaken by erythrocytes on their inner sides, these erythrocytes having a greater velocity.

It is in the large elastic vessels that the factor of diastole becomes important, for during this time, which is longer than systole, the velocity of the blood must be considerably reduced; in fact in places in the aorta the blood probably reverses its direction. This marked slackening of velocity, less noticeable in the arteries further from the heart, means a reduction in the force drawing the cells toward the center of the vessel. However, the velocity of the corpuscles in the blood would not be reduced to the same extent as that of the plasma, for the corpuscles possess inertia and for a small fraction of time would tend to continue to move in their original direction, until the next systolic impulse moves both corpuscles and plasma forward again. Thus the velocity of the blood stream, the force tending to pull corpuscles in, is reduced more than the velocity of the corpuscles, the impinging of one on the other being the force tending to drive the larger and lighter corpuscles out.

EFFECT OF THE SIZE OF CORPUSCLES

Under this head three principles must be taken into account:

1. The larger the corpuscle the greater is its surface area, and so the larger is the surface exposed to impinging erythrocytes. However, this larger surface does not make up for the increase in mass. If one corpuscle is twice the diameter of another, its surface will be increased four times, but its volume, and therefore mass, will be increased eight times.

2. The larger the corpuscle the greater will be the velocity of erythrocytes colliding with its surface nearer the center of the vessel. As the kinetic energy varies as V^2, and as V is the difference in velocity between the leukocyte and the bumping erythrocyte, the larger leukocyte will have packets of much greater energy tending to drive it out.

3. The larger the corpuscle the more often it will be bumped on its inner side, for these erythrocytes are traveling with greater velocity and will thus be passing by more frequently.

The sum of 1, 2 and 3 means that, although larger and thus of greater mass, the larger lipophages quite possibly would receive vastly increased complements of energy tending to drive them out. Finally, the larger lipophages, because of their greater mass and hence greater kinetic energy, would bump away more easily the erythrocytes that they themselves overtake.

It would be interesting to clothe the skeleton of this hypothesis with details of actual velocities, and in fact equations are available to find the velocity of any particle at a given distance from the center of a tube. It is considered that the type of flow in the aorta is turbulent, for the following reasons: 1. The diameter is relatively large. 2. The velocity is relatively great. 3. The flow is intermittent. 4. Large tributaries would tend to disturb any even flow. The equation for turbulent flow given by Kármán,[13] cited by Vejlens,[5] is

$$w = Wmax\ [1 - (\tfrac{y}{r})^2]^{1/7}$$

where

w = velocity of particle
$Wmax$ = maximum velocity of fluid
y = distance of particle from center of tube
r = radius of tube

Immediately, however, it is apparent that in the aorta there are factors introducing a large margin of error into the use of this equation: 1. The velocity in the aorta differs from systole to diastole. 2. The diameter of the aorta is greater in systole than in diastole. 3. The region in the aorta where the axial zone passes into the peripheral zone, i. e., where these collisions in which one is interested occur, is unknown. Vejlens [5] stated that the peripheral zone is always 1 red cell diameter wide, irrespective of velocity or size of vessel. Sandison [14] expressed the belief that the peripheral zone is larger the swifter the stream. Perhaps it varies with systole and diastole. For the purpose of this illustration it is considered as 7 microns. 4. The velocity of each corpuscle is considered to be that of the stream of plasma corresponding with the center of the corpuscle, an assumption by no means necessarily true. 5. The diameter of the aorta varies with age and so also the velocity in the vessel.

Accepting the premise that if one considers the aorta as a rigid tube with a stream of constant velocity one is inviting a large margin of error except perhaps for a short period in the cardiac cycle when that diameter and velocity obtain, one may indicate the velocity of the corpuscles concerned as shown in figure 2 for each cell.

For convenience, erythrocytes 7 microns in diameter are shown as circular. Cell A, 12 microns in diameter, is a polymorphonuclear leukocyte. Cell B, 20 microns in diameter, is a lipophage. The remaining cells are erythrocytes. Velocities are expressed in millimeters per second to the nearest tenth of a millimeter. The maximum velocity

13. Kármán, V.: Ztschr. f. ang. Math. u. Mekanik. **1**:233, 1921; cited by Vejlens.[5]

14. Sandison, J. C.: Anat. Rec. **54**:105, 1932.

of the aortic blood stream is taken to be 300 mm. per second (Cowdry [15]), and the diameter of the aorta to be the average at age 50 found by Kaufman and Aschoff, cited by Krafka,[16] i. e., 22 mm.

It is apparent that erythrocyte 2 is advancing on polymorphonuclear cell A with a velocity of 5.5 mm. per second (131.9 — 126.4). Erythrocyte 6 is advancing on lipophage B with a velocity of 7 mm. per second (138.4 — 131.4). Since kinetic energy varies as V^2, corpuscle B receives an impulse about 1.6 times that received by corpuscle A with respect to the erythrocytes mentioned. Also, erythrocyte 6 is advancing on corpuscle B with velocity 1.3 times as great as that with which E 2 is advancing on A; therefore B will receive 1.3 times the number of impulses—presuming erythrocytes follow each other in series. It will receive in addition the contacts of erythrocyte 5, which, however, in this case will be of relatively low kinetic energy. Corpuscle A itself bumps erythrocyte 7, and corpuscle B bumps erythro-

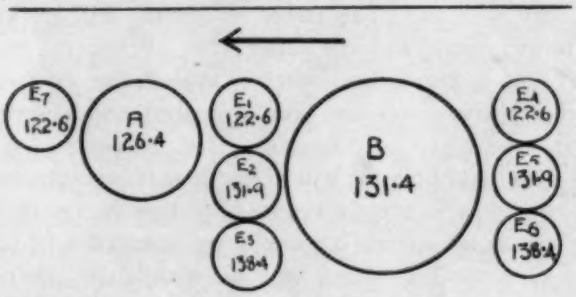

Fig. 2.—Blood stream velocities of a polymorphonuclear leukocyte (*A*), a lipophage (*B*) and erythrocytes (*E 1* to *6*).

cyte 1, but corpuscle B, because of its larger mass and greater velocity, has at least twenty times the kinetic energy of polymorphonuclear cell A with respect to the erythrocytes concerned; therefore it pushes the erythrocyte aside more easily. All these points demonstrate that the larger the cell the more easily it is extruded. It must be remembered also that cell B has approximately three times the surface area of cell A, allowing three times the space for collisions. Against all these factors, however, it has nearly five times the inertia.

At first it may not seem significant that an erythrocyte should overtake and collide with another corpuscle at a velocity of 5 mm. per second. However, for such a small body this is a great velocity; using common methods of analogy one finds that the erythrocyte 7

15. Cowdry, E. V., in Arteriosclerosis, New York, The Macmillan Company, 1933.

16. Krafka, J.: Arch. Path. **20**:81, 1935.

microns in diameter can pass 500 polymorphonuclears 10 microns in diameter laid side by side in one second, and a bumped corpuscle can suffer several hundred impacts in the same period. However, too much stress should not be placed on the data given; in view of the mathematical and hydrodynamic difficulties of the problem they can be considered only as illustrative. As there is about 1 leukocyte to every 600 erythrocytes, it is hardly necessary to consider the effect of leukocytes colliding with erythrocytes on the distribution of erythrocytes and quite unnecessary to consider the effect of one leukocyte colliding with another.

It is apparent that in the phenomena so far described size of corpuscle is of far more importance than the relatively small differences in specific gravity. It appears, in fact, in the light of present knowledge that only when the influence of gravity is brought to bear will corpuscles of a specific gravity greater than plasma act much differently from corpuscles of a specific gravity less than plasma, as some well filled lipophages may well be. The effect of gravity will obviously be to make the former settle and the latter rise. When the current in a small vessel that is nearly horizontal is slowed, the erythrocytes tend to settle; this phenomenon has been described and photographed by Knisley and associates.[7] Presumably, also, corpuscles of a specific gravity less than that of plasma will in the same circumstances rise. The velocity in the aorta is greatly reduced in diastole; in the peripheral zone of the stream into which large cells are bounced it is much slower. The effects of several diastoles will be cumulative. Whenever the walls of the aorta are out of the vertical, this process of rising of the light particles and falling of the heavy ones will tend to bring the corpuscles into contact with the walls if the current is slow enough. Gravity will thus exert its action in that narrow region a few microns from the intima. Lipophages will tend to rise and stick to the intima, especially during diastole. It is true that erythrocytes will settle on the intima below as well, although there will not be the same tendency for them to be pushed into the peripheral zone; but this is of little consequence, as they will not stick. Beard and Rous[12] have described the stickiness of Kupffer cells in culture as far surpassing that of any other cells, including polymorphonuclear leukocytes.

It is apparent that macrophages containing many substances, occurring naturally or experimentally, can circulate in the blood. Leary[11] mentioned silica and silicates, carbon, magnesium dioxide, lapis lazuli, mercuric sulfide, colloidal dyes, bacteria and blood pigments. These cells, with the exceptions described later, never appear in the intima; only cells containing lipids do so. Leary suggested that this difference is due to chemotaxis. I suggest it is due to specific gravity. The

exceptions are the macromolecular substances of Hueper,[17] and it would be interesting if one could be informed of their specific gravity, remembering, however, that injection of these substances may well change the suspension stability of the blood, causing aggregation (or sludging) of erythrocytes, thus enormously increasing the mass of these cells as compared with leukocytes.

It is also necessary to call attention to the report of Katz and Dauber[18] that in cholesterol-fed animals the intimal capillaries of the aorta were crammed with lipophages. Why should this be so, unless the peripheral stream of the aorta contained chiefly lipophages? Chemotaxis here seems unnecessarily speculative. Why should the peripheral stream then be full of lipophages and not full of the more numerous polymorphonuclear leukocytes? Again it seems that size and specific gravity appear the only satisfactory answers.

Unfortunately, the problem is by no means as simple as I have demonstrated. It is in fact of the greatest complexity, and a full analysis of all the factors concerned will require the application of the finest mathematical minds to a difficult problem of hydrodynamics. In considering the reaction of one cell with another it will be necessary to include the effect of viscosity, the effect of the shape and the lie of erythrocytes at the time of impact, the differential effect of the impact according to the position of the erythrocyte on the circumference of the leukocyte (i. e., the more tangential the blow the less the bumped cell will be pushed forward and the more it will be pushed aside) and the variations in size of the vessel and in velocity of flow with the cardiac cycle. Also to be considered are the kinetic energy of the particles of plasma themselves as they strike the corpuscle, and the electromagnetic attraction and repulsion of corpuscles, one with another. Finally, there may be other factors, as yet undescribed, that may influence the position of corpuscles in the blood stream, and dogmatism such as Fahrens[4] and Vejlen[5] have shown in regarding the size of the corpuscle as the only significant factor is quite unjustified.

SUMMARY AND CONCLUSIONS

The long-established theory that white corpuscles move from the axial to the peripheral stream of the blood by virtue of their lower specific gravity is reviewed in the light of the work of Fahreus and Vejlens in which this process is denied. It is pointed out that as nearly half of the volume of the blood consists of red blood corpuscles, and as these corpuscles move with differing velocities according to their distances from the intima, a series of collisions must ensue when erythro-

17. Hueper, W. C.: (*a*) Am. J. Path. **18**:895, 1942; (*b*) **21**:1021, 1945; (*c*) Arch. Path. **39**:117, 1945; (*d*) **41**:130, 1946.

18. Katz, L. N., and Dauber, D. V.: J. Mt. Sinai Hosp. **12**:382, 1945.

cytes or erythrocyte clumps overtake leukocytes. The result of these collisions is to force the leukocytes into the peripheral stream. The mass of the corpuscle, which is the product of its volume and specific gravity, and the relative velocities of the corpuscles concerned, are the main relevant factors that decide to what extent this "bouncing out" will occur. Evidence is brought forward to the effect that the lipophage will have a specific gravity less than that of other leukocytes, and perhaps even less than that of plasma. The velocities are considerably reduced in the large elastic arteries during diastole, and in the slow peripheral zone and adjacent portions of the axial stream gravity may also have some influence in bringing these lipophages into contact with the intima, facilitating their entrance therein and so producing the earliest lesion of atheroma.

INFLUENCE OF LOCAL ACIDIFICATION OF TISSUE BORDERING CANCEROUS GROWTHS

With Special Reference to the Eosinophil, the Paneth Cell and the Acidophilic Plasma Cell

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THIS paper is an outgrowth of a protracted search to determine, in the first place, why cancer of the small intestine is so uncommon; in the second place, why cancer when it does arise in the small intestine tends to grow so slowly and metastasize so late, and, in the third place, why intramural extensions of carcinoma of the small intestine are so seldom found. The low incidence of carcinoma of the small intestine, in striking contrast to the abrupt increase in that of carcinoma of the stomach above and that of carcinoma of the large intestine below, has provoked the interest of pathologists for many years. The infrequency of carcinoma of the small intestine has been attributed to various factors, such as the absence of anatomic constrictions like the pyloric and ileocecal valves, the absence of abrupt changes in structural continuity and the minimal degree of chronic irritation in this portion of the intestine. Still, none of these explanations seems wholly adequate to account for the low incidence and peculiar growth of carcinoma of the small intestine.

It is significant that for nearly a century pathologists have noted peculiar collections of acidophilic cells located in the immediate vicinity of cancerous growths, particularly in connection with carcinoma of the gastrointestinal tract. These acidophilic cells include the eosinophilic granulocyte and the plasma cell, both of which are normally numerous in the lamina propria of the small intestine, and the Paneth cell, present in the bottoms of the crypts of Lieberkühn of the small intestine. Although knowledge of the function of these three groups of cells is appallingly meager, the fact that they are occasionally seen arranged about cancerous growths, oftentimes forming a virtual barrier against the advancing tumor, is significant enough to merit considerable investigation.

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EOSINOPHILS

Eosinophils are observed in many conditions, such as acute and subacute inflammation, allergic states, parasitic infestation and cancer, but there is no satisfactory explanation for this empiric observation. Eosinophils appear most conspicuous in inflammatory zones at a time when the host appears to be successfully combating the invader. For this reason, the local appearance of eosinophils is regarded as a favorable prognostic sign. One does not encounter local eosinophilia in every cancer examined or in every region of the same neoplasm. Eosinophils are observed most commonly about cancer in areas where the host appears to be waging a successful battle against invasion, as evidenced by disintegration and fragmentation of carcinoma cells associated with early local defensive fibrosis. Generally one does not see eosinophilia about old, inactive, scirrhous carcinoma. In certain carcinomas, particularly those of the gastrointestinal tract, eosinophils are exceptionally common. Biggart [1] observed local eosinophilia about every neoplasm of the gastrointestinal tract that he examined, regardless of the presence or the absence of secondary inflammatory changes.

Although the eosinophil is a conspicuous cell of the blood, the bone marrow and some connective tissues, its origin remains obscure. In 1880 Ehrlich [2] proposed the theory that there is a single and distinct strain of eosinophils, formed in the bone marrow, which are disseminated to all parts of the body by the circulating blood. Weidenrich [3] maintained that there is no true strain of eosinophilic cells, believing that the cytoplasmic granules are phagocytosed particles originating from hemoglobin. Rous [4] regarded the typical granules as protein absorbed from the intestine. Brown [5] considered these granules as toxic substances absorbed by neutrophils. A third opinion, supported by Downey,[6] distinguishes two types of eosinophilic cells. He considered the circulating eosinophils as developing from myelocytes and the tissue eosinophils as of local origin.

The theory of Ehrlich has gained considerable support. In 1932 Biggart [1] concluded from his studies of the eosinophils of normal and pathologic tissue that there is no distinguishing feature between those of the fixed tissues and those of the circulating blood. The mature eosinophil, with its bilobed nucleus and coarse, highly refractive granules, is common to both locations. The cytoplasmic granules give identical oxidase and peroxidase reactions, stain electively with

1. Biggart, J.: J. Path. & Bact. **35**:599, 1932.
2. Ehrlich, P.: Ztschr. f. klin. Med. **1**:553, 1880.
3. Weidenrich, F.: Anat. Rec. **4**:317, 1910.
4. Rous, F. P.: J. Exper. Med. **10**:537, 1908.
5. Brown, T.: Bull. Johns Hopkins Hosp. **73**:79, 1897.
6. Downey, H.: Folia haemat. **19**:148, 1915.

acid dyes and contain ionizable iron. Through vital staining the eosinophils are found to be actively motile, propelled by means of cytoplasmic pseudopodia. According to Jacobsthal,[7] the eosinophils are capable of extruding their granules.

Eosinophils are frequently seen along the margins of neoplasms and in the connective tissue stroma of carcinoma. Figure 1*a* shows the local eosinophilia encountered at the spearhead of an advancing carcinoma of the rectum. The carcinoma cells in this region show disintegration and fragmentation, associated with early local connective tissue fibrosis. This area of eosinophilia is literally sprinkled with extracellular small uniform round eosinophilic granules, comparable to the cytoplasmic granules of the mature eosinophil. Moreover, this area stained more intensely with eosin than some of the nearby areas where the deeply basophilic carcinoma was obviously showing rapid growth. No recognizable carcinoma cells were seen in the immediate area of the eosinophilia.

PANETH CELLS

The acidophilic Paneth cells were first described by Schwalbe[8] in 1872. Paneth[9] in 1888 brought these cells to the attention of investigators, and they are now recognized as constant constituents of the glands of the small intestine. The incidence of the Paneth cells is greatest in the ileum and the jejunum, where they are about equal in distribution, while they occur less frequently in the duodenum and the appendix. It is estimated that there are about twenty Paneth cells in each crypt, and there are estimated to be four to five million crypts, which would make an estimated total of eighty to one hundred million Paneth cells. Paneth cells may occur occasionally in the large intestine and stomach, particularly under certain pathologic conditions. Their common location is in the fundi of the crypts of Lieberkühn of the small intestine. Paneth cells contain many uniform coarse cytoplasmic granules, which stain deeply with eosin and other acid dyes. These cells contain a spherical nucleus, which is located near the base, is poor in chromatin and usually reveals a nucleolus. The granules are located between the nucleus and the lumen of the gland of Lieberkühn.

Paneth[9] regarded these cells as a specific kind of gland cell, wholly different from the goblet cell. Klein[10] in 1906 found that the cells responded to physiologic stimulation, a finding which led him to consider that they represent a zymogenic cell involved in digestion. By differential staining, Dunn and Kessel[11] indicated that the Paneth

7. Jacobsthal, E.: Virchows Arch. f. path. Anat. **234**:12, 1921.
8. Schwalbe, G.: Arch. f. mikr. Anat. **8**:92, 1872.
9. Paneth, J.: Arch. f. mikr. Anat. **31**:113, 1888.
10. Klein, S.: Am. J. Anat. **5**:315, 1906.
11. Dunn, T., and Kessel, A.: J. Nat. Cancer Inst. **6**:113, 1945.

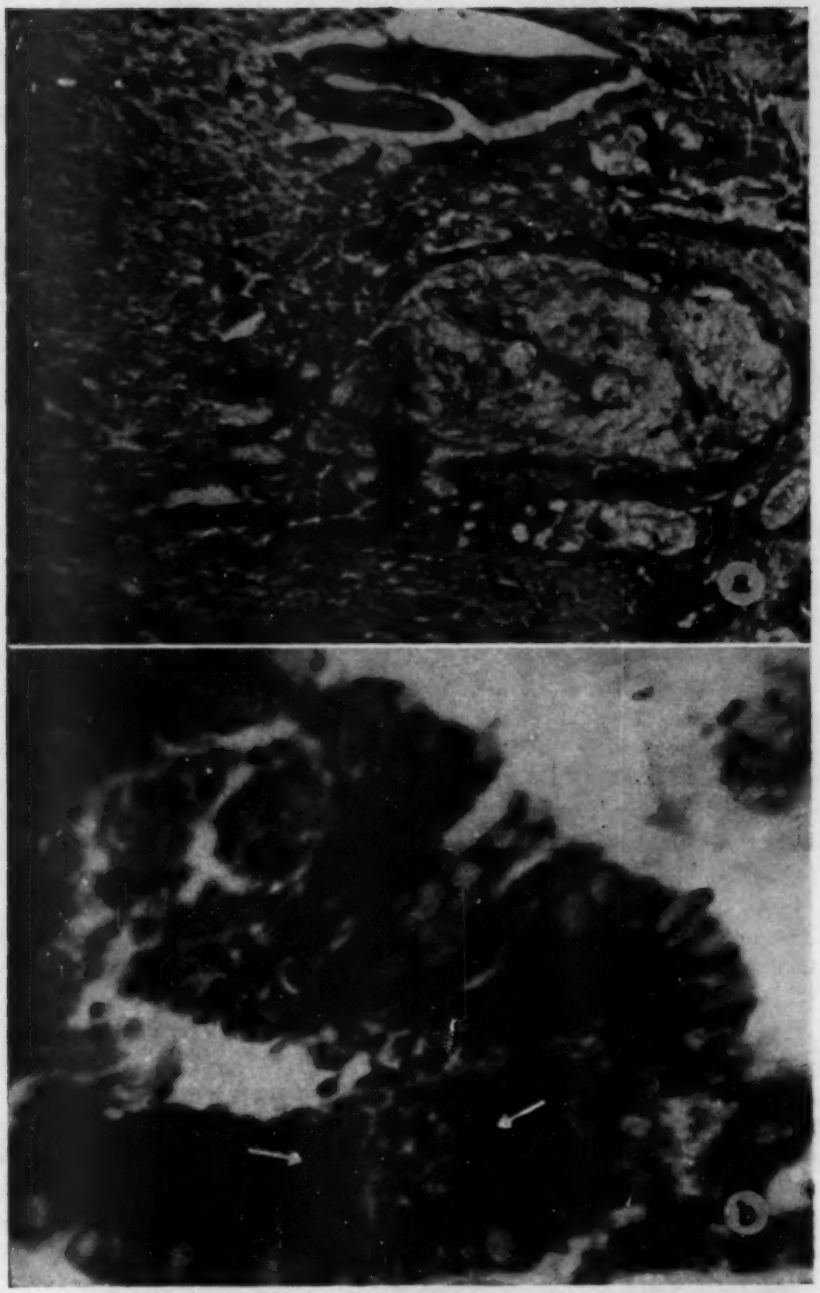

Fig. 1.—An advanced adenocarcinoma of the ampulla of the rectum in a white man aged 56: (*a*) The spearhead of the advancing carcinoma is shown where marked local eosinophilia was encountered, accompanied by dispersed extracellular eosinophilic granules. The foremost carcinoma cells show fragmentation and disintegration; eosin-methylene blue; × 1,872.5. (*b*) Paneth cell hyperplasia occurring in the adenocarcinoma; eosin-methylene blue; × 1,872.5.

cell granules contain a zymogenic substance. These granules accumulate during starvation and disappear during digestion. As shown in figure 2, these granules are occasionally found in the lumens of the crypts of Lieberkühn, and the cells can therefore be interpreted as having a secretory function in digestion. Herzog [12] observed that when Paneth cells exist in the stomach or the large intestine in association with carcinoma the local area is more eosinophilic, indicating local acidification of the tissue.

The acidophilic Paneth cell and carcinoma have an interesting relationship in regard to incidence. Where Paneth cells are numerous, as in the small intestine and the appendix, carcinoma is uncommon. We have found Paneth cells to be normally numerous in the unaltered mucosa of the appendix, as shown in figure 2.

Fig. 2.—Appendix vermiformis of a white man, showing Paneth cells with some of their granules extruded into the lumen of the gland; eosin–methylene blue; × 1,872.5.

Abnormal Paneth cell proliferation is encountered in connection with carcinoma of the stomach and with carcinoma of the large intestine. Paneth cells are seldom found normally in either of these locations. In figure 1 *b* hyperplasia of Paneth cells is seen in connection with carcinoma of the rectum. Paneth cell proliferation in Brunner's gland is frequently observed in cases of carcinoma of the stomach. Normally only an occasional Paneth cell is found in Brunner's glands of the duodenum.

ACIDOPHILIC PLASMA CELLS AND RUSSELL BODIES

Near the turn of the century the large globoid acidophilic bodies (Russell bodies) often seen in the region of certain cancers attracted

12. Herzog, A.: Am. J. Path. **13**:351, 1937.

much interest and provoked much discussion. These peculiar round acidophilic bodies were noted as early as 1858 by Fox.[13] In 1890 Russell[14] fully described them. He recognized them as "cancer parasites" of blastomycetic nature. In 1901 Hektoen and Reisman[15] accurately defined Russell bodies as "peculiar, globular, homogeneous, extra-cellular and intra-cellular formations of varying size; frequently they are aggregated in mulberry-shaped conglomerations. They stain red with acid fuchsin and deep blue with the Gram-Weigert stain. These bodies occur in many normal tissues and in a great variety of pathologic processes, a favorite place for their study being glandular proliferations of the mucous membrane of the stomach (Hansemann, Thorel)."

According to Schridde[16] the majority of plasma cells contain Altmann granules, while a smaller number possess fine bluish gentianophilic granules, the precursors of Russell bodies. These gentianophilic granules, through growth and confluence, give rise to the larger and more characteristic Russell bodies. With Pappenheim's differential staining method employing safranin and methyl green, Russell bodies stain red in a background of green. These bodies have a strong affinity for the acid dyes, staining red with acid fuchsin or eosin. Their staining reactions indicate that they are homogeneous and semifluid in character, being composed of a hydrophilic acid-like protein substance.

There has been much controversy over the significance of Russell bodies. Most workers regard them as degenerative products of the plasma cell, while some workers believe they are secretory in nature. Downey[17] in 1911 and Kingsley[18] in 1924 presented the view that the Russell body is probably a normal secretion of the plasma cell. In 1931 Michels[19] presented a comprehensive review of the morphologic aspects, the function and the development of the plasma cell, including Russell bodies. His summary of the theories concerning the function of the plasma cell is as follows: (1) absorption of necrotic chromatin material; (2) metabolism of nuclear material; (3) physiologic secretion; (4) production of antibodies; (5) phagocytosis. There is evidence that the acidophilic plasma cell and the associated Russell bodies, in particular, have a secretory function. In certain situations these acidophilic cells appear to be playing a role of defense against the invader.

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13. Fox, W.: Med.-Chir. Tr., London **41**:361, 1858.
14. Russell, W.: Brit. M. J. **11**:1356, 1890.
15. Hektoen, L., and Reisman, D.: American Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1901, p. 93.
16. Schridde, H.: Pathologische Anatomie, Jena, Gustav Fischer, 1921.
17. Downey, H.: Folia haemat. (Teil 1) **11**:275, 1911.
18. Kingsley, D.: Anat. Rec. **29**:1, 1924.
19. Michels, N.: Arch. Path. **11**:775, 1931.

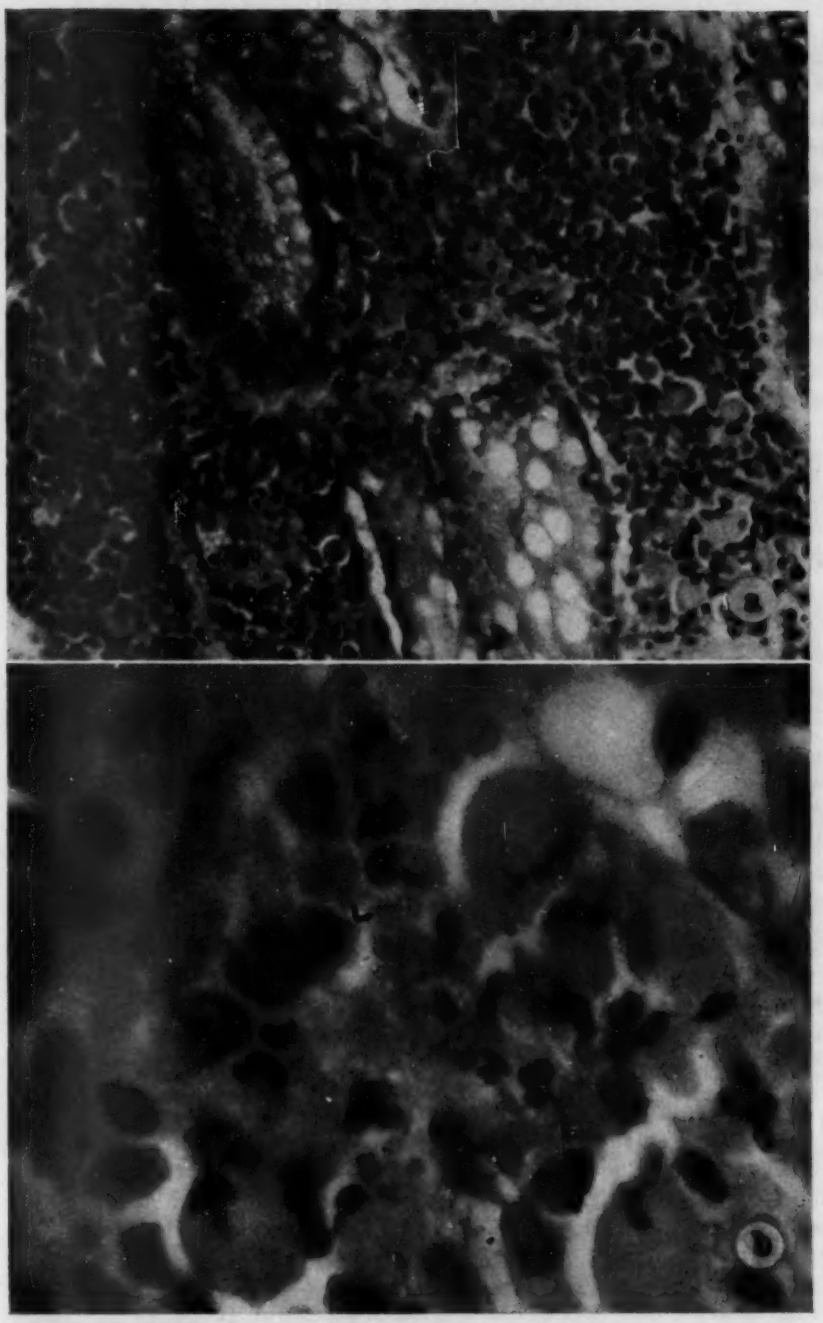

Fig. 3.—A highly invasive, poorly differentiated cell adenocarcinoma of the pylorus occurring in a white woman aged 22: (*a*) junction of the pylorus and the duodenum at the pyloric fold, showing acidophilic plasma cells and Russell bodies surrounding the duodenal glands adjacent to the carcinoma; hematoxylin and eosin; × 350. (*b*) The same field under higher magnification; hematoxylin and eosin; × 1,900.

The acidophilic plasma cell reaction with Russell body formation found about neoplasms, as described by many early workers, such as Lubarsch,[20] Saltykow,[21] Thorel,[22] Schridde[23] and Fabian,[24] is shown in figure 3 *a* and *b*. In the case illustrated, pyloric carcinoma of the stomach is sharply delineated at the junction of the duodenum. Great numbers of large acidophilic plasma cells and Russell bodies are seen densely packed about the duodenal glands, immediately adjacent to this neoplasm. No carcinoma cells are found on the duodenal side among the acidophilic plasma cells. An area under higher magnification in the same field next to the carcinoma on the duodenal side, demonstrating large acidophilic plasma cells and eosinophils bordering the neoplasm, is shown in figure 3 *b*.

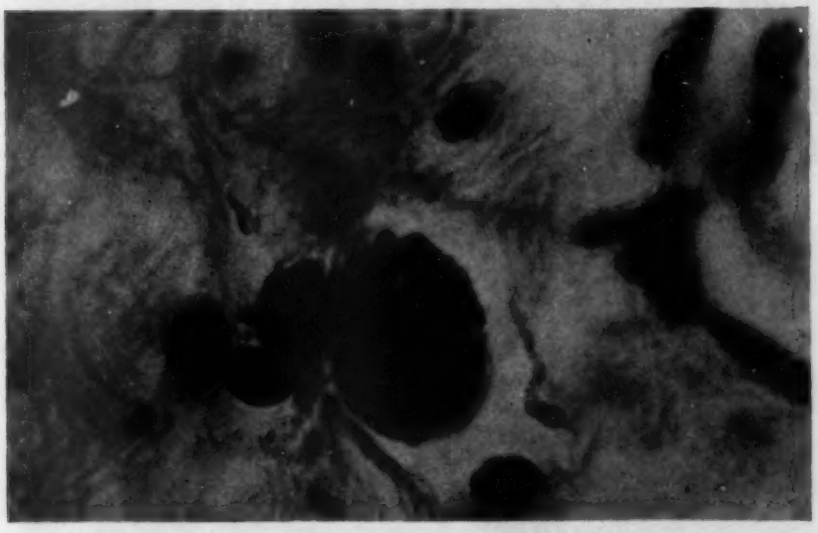

Fig. 4.—An advanced, ulcerating adenocarcinoma of the sigmoid colon densely adherent to a loop of the ileum of the small intestine occurring in a white woman aged 60. A group of acidophilic plasma cells are seen in the wall of the ileum located in the immediate vicinity of the carcinomatous attachment; hematoxylin and eosin; × 1,872.5.

Secondary carcinoma seldom invades the wall of the small intestine, even when the neoplasm is attached to the serosal surface. The photomicrograph shown in figure 4 was taken from a section through the wall of the ileum where an advanced, ulcerating adenocarcinoma of the large intestine was densely adherent to it. Large numbers of acido-

20. Lubarsch, O.: Ergebn. d. allg. Path. u. path. Anat. **12**:180, 1895.
21. Saltykow, S.: Virchows Arch. f. path. Anat. **153**:207, 1898.
22. Thorel, C.: Virchows Arch. f. path. Anat. **151**:319, 1898.
23. Schridde, H.: Arch. f. Dermat. u. Syph. **73**:107, 1905 .
24. Fabian, E.: Centralbl. f. allg. Path. u. path. Anat. **18**:689, 1907.

philic plasma cells and Russell bodies were found in the wall of the small intestine immediately adjacent to the carcinomatous attachment. A group of these large acidophilic plasma cells is shown in figure 4. Although this carcinoma was widely invading the wall of the sigmoid colon, it had scarcely penetrated the wall of the adherent loop of the ileum.

COMMENT

Observations have been presented showing that an acidophilic inflammatory cell response is made to invading carcinoma of the gastrointestinal tract. Many workers believe that the inflammation associated with neoplastic processes may represent resistance of the host. Ewing [25] emphasized that an inflammatory reaction frequently meets the invasion of tumor cells. He regarded it as a highly significant feature of cancerous conditions and stated that it must be regarded as a defensive process. Goforth and Snoke [26] expressed the belief that the many eosinophils accumulated locally about cancerous growths constitute body resistance. They concluded that the eosinophils present about carcinoma of the uterine cervix are of good omen. Schoch,[27] in studying a large group of cases of carcinoma of the cervix, found local eosinophilia in 40 per cent of the cases in which the patient survived for a five year period. Pavlovsky and Widakowich [28] also expressed the belief that local eosinophilia is a protective measure against cancer invasion. Gill,[29] in reviewing local eosinophilia in cases of cancer, concluded that abundant local eosinophilia is of good prognostic import and probably represents better than usual resistance to the advance of the neoplasm.

As previously mentioned, the function of the eosinophil is obscure. In general, an inflammatory reaction follows a well delineated pattern. As originally shown by Menkin,[30] in an inflamed area the local hydrogen ion concentration of the tissue is at first alkaline but becomes progressively acid as the inflammation proceeds. As the p_H changes, the type of inflammatory cell changes. Polymorphonuclear leukocytes flourish in an alkaline medium. According to Menkin,[31] with the local rise in acidity (p_H 6.5 or below) the neutrophilic polymorphonuclear leukocytes become injured and quickly disappear, leaving macrophages, eosinophilic cells, lymphocytes and plasma cells unimpaired. It is generally recognized by students of inflammation, that local acidification of the inflamed area is one of the important tissue defense reactions

25. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 35.
26. Goforth, J., and Snoke, P.: Am. J. M. Sc. **175**:504, 1928.
27. Schoch, E.: Zentralbl. f. Gynäk. **50**:2895, 1926.
28. Pavlovsky, A., and Widakowich, V.: Semana méd. **1**:1265, 1926.
29. Gill, A.: J. Lab. & Clin. Med. **29**:820, 1944.
30. Menkin, V., and Warner, C.: Am. J. Path. **13**:25, 1937.
31. Menkin, V.: Arch. Path. **41**:376, 1946.

against invading bacteria and other injurious agents. It is quite conceivable that certain acidophilic cells, such as the eosinophilic cell, may play an important function in acidifying the tissue, making it untenable for such injurious agents as bacteria or cancer cells through the dispersement of eosinophilic granules.

As the repair stage of inflammation approaches, the number of polymorphonuclear neutrophils becomes reduced and that of the polymorphonuclear eosinophils, plasma cells and lymphocytes is increased. Kirk,[32] in reviewing the clinical significance of eosinophilia, expressed the belief that the local appearance of eosinophils is an allergic response to sensitization of tissues which have absorbed the split proteins associated with local necrosis. It would seem easier to interpret the eosinophil as a defense cell in contrast to a scavenger cell. In the interpretation of cytoplasmic inclusions,[33] the fact that the eosinophilic cytoplasmic granules of the polymorphonuclear eosinophil are regularly round to oval, stain uniformly, are uniform in size, possess a strong affinity for the acid stains and are not associated with degenerative changes of the cell indicates that the granules are more likely secretory in nature. This belief is further supported by the fact that eosinophils do extrude their granules in particular locations. Eosinophils are not characteristically found in the immediate vicinity of suppurating inflammatory processes, but instead are usually in the background bordering the inflammatory or the neoplastic process. They are characteristically found in situations where the tissue responses appear to be winning the battle against the invader.

Many tissue stains, particularly eosin, are virtual acid-base indicators. By means of the modified eosin–methylene blue method,[34] with the eosin adjusted to a p_H 3, the eosinophilic granules stain best, indicating that they are strongly acid. In areas where these eosinophilic granules are present the whole field stains more acid. This is also true in locations where there are large numbers of acidophilic plasma cells, Russell bodies and Paneth cells.

It is curiously interesting that carcinoma of the stomach rarely extends beyond the pyloric fold into the duodenum. It is also interesting that this is the site where large aggregates of acidophilic plasma cells, Russell bodies and eosinophils occur, appearing as defenders on the side of the duodenum, as shown in figure 3. Hyperplasia of the Paneth cells is frequently encountered in this same region in cases of carcinoma of the stomach.

The appendix, like the small intestine, is rarely the site of a primary carcinoma, in contrast to the common occurrence of carcinoma of the

32. Kirk, R.: J. Lab. & Clin. Med. **23**:1137, 1938.
33. Black, C.: J. Infect. Dis. **67**:42, 1940.
34. Stovall, W., and Black, C.: Am. J. Clin. Path. **10**:1, 1940.

large intestine, which is puzzling when one considers that the vermiform process is anatomically a part of the large intestine. From the standpoint of irritation the appendix is as vulnerable if not more vulnerable than the large intestine itself. The appendix is different from the large intestine inasmuch as it normally contains many eosinophils and plasma cells in the lamina propria and many Paneth cells in the crypts of Leiberkühn. It is strikingly similar to the small intestine in this respect. That these acidophilic cells occur normally and prominently in regions of the intestinal tract where primary or secondary carcinoma is uncommon seems more than a coincidence. It is quite conceivable that these cells play a role in maintaining a certain hydrogen ion concentration that makes the soil of these regions unsuitable for the development and growth of carcinoma.

It is generally presumed that the secretions of the small intestine are entirely of an alkaline nature. Robinson, Luckey and Mills [35] demonstrated that the small intestine throughout its length adjusts the hydrogen ion concentration of its contents to fit a definite pattern. They found by experimentation that an acid-base adjustment occurred in the contents of the small intestine from higher or lower prevailing values to those values characteristic of different portions of the intestine. McGee and Hastings [36] have made the suggestion that there are cells in the intestine which can secrete acid, and others which can secrete base. It is likely that the Paneth cells of the small intestine have such an acid-secretory function.

It is interesting that the acidophilic cells are seldom the source of primary neoplasms. Although Dunn and Kessel [11] interpreted a neoplasm occurring in a mouse as a Paneth cell carcinoma, no cases have been reported in which such a tumor was observed in human beings. It is also interesting in this connection that Foot [37] noted that carcinoma of the stomach composed of eosinophilic parietal cells is not encountered; most of the neoplasms arise in the chief cells of the gastric glands.

Local acid-base equilibrium and the development of cancer would seem to warrant much investigation. To transgress into the realm of speculation, the two organs of the body that manifest a constant acid-base flux, the stomach and the lungs, are the two sites of the whole body in which carcinoma most commonly develops. Moreover, the fact that achlorhydria is common enough in cases of carcinoma to be used as a diagnostic test adds further significance to the acid-base idea. The fact that achlorhydria is not always present in gastric carcinoma

35. Robinson, C.; Luckey, H., and Mills, H.: J. Biol. Chem. **147**:175, 1943.

36. McGee, L., and Hastings, A.: J. Biol. Chem. **142**:893, 1942.

37. Foot, N.: Pathology in Surgery, Philadelphia, J. B. Lippincott Company, 1945, p. 222.

does not necessarily discredit this possibility. It is conceivable that an acid-base disturbance could occur in a small area of the stomach without appreciably altering the free hydrochloric acid secreted into the stomach as a whole. It is quite probable that local alkalinization of the tissue for a long period, as in instances of old chronic catarrhal inflammation of the lungs, the stomach, the large intestine or the uterine cervix, could be an important predisposing factor in the development of carcinoma.

SUMMARY

Photomicrographs are presented showing eosinophilic cell, Paneth cell and acidophilic plasma cell reactions about carcinoma of the gastrointestinal tract. Local acidification of the tissue is found where these three groups of cells are increased, as indicated by the eosinophilic reaction of the tissue. Carcinoma cells are shown to be fragmenting and disintegrating in these acidophilic areas. It is concluded that local acidification of the tissue is a defensive response of the host. Further, it seems plausible that the eosinophils, the Paneth cells and the acidophilic plasma cells commonly occurring in the small intestine and the appendix could be an important factor in explaining why cancer is so uncommon and tends to grow so slowly and metastasize so late in this region of the gastrointestinal tract. Moreover, the large numbers of acidophilic cells occurring in the region of the pyloric fold may explain why carcinoma of the pylorus of the stomach seldom extends beyond the pyloric fold into the duodenum.

POSTMORTEM EXAMINATION OF TEETH AND SUPPORTING STRUCTURES TO AID IN PERSONAL IDENTIFICATION

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AS LONG as wars, explosions, fires, murders or other kinds of major or minor catastrophes persist, the need for establishing the personal identities of death victims will be present. In this paper I shall attempt to demonstrate various specific methods and means, as well as some general considerations in the dental field, which can be used for the purposes of identification.

The recent war has demonstrated how the bodies of hundreds of men, women and children have been identified wholly or in part by their teeth and supporting structures. Ström [1] described how the remains of 211 Norwegian patriots, murdered by the Nazis, were identified. These bodies were buried in a common grave and all were destroyed beyond recognition. Since none could be identified by physical appearance, the jaws were sawed off and kept in specially labeled jars. Through the cooperation of Norwegian dentists who furnished dental records, 156 of these bodies were identified on dental evidence. The roentgenogram was valuable in this particular examination.

Another case was that of a Norwegian army plane that had been shot down in flames. The bodies of the two officers were charred beyond recognition. One of the fliers was positively identified by a metal post that was found, undamaged by the heat, in the root canal of a tooth where it had supported an anterior crown. Thus identity was established on the basis of a detailed dental record.

In another article Ström [2] referred to the identification of 25 victims, burned beyond recognition in a fire in Oslo, Norway, in 1938. All of the bodies were positively identified, 6 entirely on the basis of dental records. In 10 of the cases the dental examination was the most reliable evidence, with other factors aiding in the identification. In the remaining 9 cases no dental records were available, and identity was established by other means. Without the dental evidence, however, it would have been impossible to establish positive identifications of many of these victims.

From the Harvard School of Dental Medicine.

1. Ström, F.: Norske. tannlaegeforen. tidende **56**:153, 1946A.
2. Ström, F.: Odont. tidskr. **54**:443, 1946.

Perhaps one of the most well known cases of identification is that of a Dr. Parkman who was murdered about one hundred years ago by a Dr. Webster. Identity was established mainly by the finding, among badly charred bones and ashes of the victim's body, three artificial porcelain teeth and a few grains of melted gold. The remnants of the bones of the lower jaw were fitted together and the victim's dental surgeon was able to identify his earlier patient with certainty by means of a peculiar depression on the left side of the lower jaw and the remnants of the artificial dentures.

In each of the cases a previously made-out dental record was instrumental in establishing identity. If no previous chart of a dental examination were available, what general information might one gather from a careful examination of the teeth and the jaws of the dead person?

The question of establishing the age of the dead person is always of utmost importance. Age may be determined rather accurately by the various stages of calcification that one finds in the crowns and the roots of the teeth. Because these teeth may be both erupted and unerupted, a roentgenogram is essential in an examination of this type. Calcification of crowns of deciduous teeth begins in utero at the embryonal age of 4 to 5 months. At 6 months in utero all deciduous teeth have begun to calcify, and at birth all deciduous teeth are in various stages of development. In addition, calcification of the four first permanent molar teeth is usually just beginning. The approximate times at which the deciduous teeth erupt into the oral cavity are as follows:

Central incisors	7 mo.
Lateral incisors	9 mo.
First molar and canine	12-16 mo.
Second molar	2 yr.

After the eruption and subsequent loss of the deciduous teeth, one sees the advent of the permanent teeth. These teeth also calcify and erupt at rather definite ages, so that by examining (grossly and roentgenologically) as little as one half of a jaw of the dead person, one may procure valuable information as to his aged. Roughly, the times of eruption of the permanent molar teeth are as follows:

First molar	6 yr.
Second molar	12 yr.
Third molar	17-21 yr.

Intermediary age levels may also be determined if a quadrant of the jaw is available. That is, at 9 years of age one should find 12 permanent teeth in the mouth: 8 incisors, i. e., 2 in each quadrant of the jaw, and 4 molars, i. e., 1 in each quadrant of the jaw. Normally at this age the deciduous molar and canine teeth should also be present.

At 11 years of age one should find 20 permanent teeth in the mouth: 8 incisors, 8 premolars and 4 molars.

At 13 years of age one should find 28 permanent teeth in the mouth, and no deciduous teeth remaining: 8 incisors, 8 premolars, 4 canines and 8 molars.

At approximately 8 to 10 years of age, calcification begins in the crown of the third molar tooth. The root ends of this tooth should normally be finally calcified and fully formed at about 25 years of age. Any intermediary age may thus be determined by a roentgenogram of the area of the third molar tooth either before or after the eruption of the tooth.

Beyond the age of 25 years, after complete calcification of the ends of the roots of the third molar tooth, it becomes increasingly difficult to determine the age of the patient by inspecting the teeth or even by roentgen examination. A few observations may be noted, however. After the complete root length has been reached, a determination, by roentgenogram, of the relative size of the pulp chambers of the teeth is important. In a young person little secondary dentin has been deposited, and hence there is a large pulp chamber. In an old person the pulp chamber often may be practically obliterated by the secondary dentin that has been deposited over the years. This knowledge is helpful in determining age.

After a person is 30 years of age caries often begins to develop in the cementum. In a patient whose teeth are highly susceptible to caries, accompanied by a recession of gingival tissue, one finds caries attacking the root surface of the tooth, normally covered by cementum. This type of caries is rarely found in young persons, 30 to 35 years of age, but may be extensive in patients 40 to 45 years of age or older. This is not conclusive evidence, but may possibly be of value.

Another observation is the amount of attrition, or wearing, present in the teeth. This cannot be an accurate observation, because habit and occupation, among other things, could cause teeth of a young adult to be much worn and to have the appearance of teeth of an old person. During the era of the dust bowl storms, some years ago, it was not uncommon to see young adults with teeth worn perfectly flat, with the bite closed as much as 4 to 6 mm., because of the excessive amount of fine grit and sand in the air. Generally speaking, however, the cusps of the teeth of an old adult are worn flat and smooth, while the cusps of a young person are unworn and sharp with marked interdigitation.

In an edentulous person, the general appearance of the mandible is all one has that will help differentiate a young from an old person. In the old adult one finds a generalized uniform thinning of the bony structure of the mandible. Depressions may exist in the bony structure

of the old adult which at one time may have been so-called "pyorrhea" pockets. In general, the superior and the inferior diameter of the mandible would be of most use in establishing age, the greatest diameter being associated most often with the young adult and the smallest diameter with an old adult.

Disease, characterized by loss of bone, known more commonly as "pyorrhea," may be instrumental in determining the age of the person. The type of "pyorrhea" characterized by deep, individual, soft tissue pocket formation accompanied by a loss of bone is rarely a disease of the young adult. The type of "pyorrhea" characterized by a generalized loss of bone, known as diffuse alveolar atrophy, may more commonly be seen in young adults. In general, however, "pyorrhea" is an affliction peculiar to the middle-aged or older person. A dental record with "pyorrhea" pockets accurately indicated would be valuable in establishing identity because of the inability, except in rare instances, to produce new bone in these diseased areas. Thus even years later, when all teeth were lost, these areas might still show as healed depressions in the edentulous mandible or maxilla.

To determine sex from the teeth alone is practically impossible. There is such a wide variance in the size and the shape of the teeth and supporting structures within each sex that their use to determine sex is nearly hopeless. Habits, however, might throw some light on the subject. A rather common observation is a decided groove in a central or a lateral incisor belonging to a woman who did a great amount of sewing and bit off the thread with this tooth and its opponent. A cobbler is apt to have a worn area between two teeth where he habitually holds nails. A musician, in like manner, will have definite markings on his teeth where they contact his instrument. A definite depression of a tooth, as well as a wearing away, is present in every habitual pipe smoker. Further, a characteristic black, tarry stain or residue is present on the lingual surfaces of the teeth of persons whose pipe smoking is heavy. This can be differentiated from the common brown stain caused by cigaret smoking—found usually in the lower anterior region. Thus indirectly, through habit, one may gain some information as to the sex of the dead person.

Besides age and sex, one would like to be able to determine other characteristics which might be of importance in identifying the victim of unexplained death. If a crown of an upper central incisor is available, one may obtain some knowledge as to facial contour, height and perhaps type of build from it. By inverting the tooth and allowing the incisal edge of the crown to correspond to the hair line of the owner and then looking at the labial aspect, one can roughly determine the shape of the face. Facial shapes fall into three classifications, i. e., square, tapering and ovoid. In carrying this further, the long, thin,

square crown would indicate a long, thin, square type of face; a tapering crown would indicate a face tapering markedly in the region of the chin; an ovoid crown would indicate a round-faced person. These rules are not infallible, but the resemblance is striking enough in most cases to be more than just coincidence.

Occasionally in a dental examination a hard, bony structure is discovered in the midpalatal area of the patient, the so-called torus palatinus. This bony growth may be from 5 to 25 mm. in diameter and persists throughout life. Occasionally, in like manner, hard, bony protuberances from 3 to 10 mm. in diameter may be found unilaterally or bilaterally on the inner surface of the mandible on each side of the midline. When found here they are called torus mandibularis. These develop in the young adult and persist throughout life, remaining even after the loss of all teeth unless removed surgically. If a previous knowledge of their existence has been recorded, they may contribute to identification.

Another facial characteristic which may be valuable in establishing identity is the position of the upper relative to the lower jaw. Any close friend or even casual acquaintance retains a mental picture of a general facial appearance. In an attempt at establishing identity, even if the soft tissue has been completely lost, the jaws may be reassembled in their characteristic position. This position can easily be reestablished by matching worn surfaces where the teeth contact in normal occlusion. From this reassembled articulation a description can be written which could be recognized by a member of the family, a friend or even an acquaintance. The jaws of the victim when articulated might fit in such a manner as to cause the teeth in the lower arch to project anywhere up to 25 to 30 mm. anterior to the upper arch. This of course would be in a person with a prominent chin. If the lower arch were anywhere up to 10 to 20 mm. posterior to the upper arch one would visualize a person with a marked receding chin. The normal relationship obtains when the lower teeth bite just lingual to the upper teeth with no space between them.

Other distinguishing characteristics might be: spaced anterior teeth either in the upper or in the lower arch or in both. Often these are outstanding in a person's appearance. Teeth may be beautifully in line or there may be irregularity of one or all teeth. There may be an uneven wearing of one or all of the anterior teeth. There may be areas of hypoplastic or discolored enamel, owing to a defect in enamel formation. There may be characteristic notches, grooves or fractures which would give a person a characteristic appearance. A person living in an area where fluorine is present in the drinking water in excess of 2 to 4 parts per million during the period in which his teeth

were developing would have characteristic yellowish brown pigmented areas in the enamel. If the condition is sufficiently severe, the term "mottled enamel" is applied. This characteristic mottled appearance remains throughout the life of the person and is an excellent aid in establishing identity.

It has been found further that teeth whch have developed in a high fluorine area have a much higher fluorine content than teeth which have developed in a nonfluorine area.[3] One may assume, then, that a determination of fluorine made on the ash of the tooth might help establish identity by proving that the person either lived or did not live in a particular area during the time his permanent teeth were developing.

The part that the teeth themselves play in a positive identification cannot be overemphasized. Each tooth has five surfaces, any one of which may become carious and require dental attention in the form of a restoration or restorations. Thus a combination of restorations may be present in any given mouth at any time. In addition, various types of restorative materials are routinely used, which would further individualize a mouth so that it could be distinguished from another.

Also, inlays, crowns and fixed bridges might be present in a particular mouth, the outline, the contour and the construction of which would be peculiar to that mouth alone. Suppose that a partial denture was present. If one considers individual variations as to tooth size and shape, tissue form, bone contour and arch size and shape, it becomes apparent that a partial denture could not possibly fit any mouth but the mouth of the person for whom it had been constructed.

Granted, then, that no two human dentitions are identical, one sees the immense value of adequate records made by practicing dentists and the necessity that they be available at all times for the purposes of identification.

If dental records are not available, a careful examination of the remaining teeth may still give much information. Dental practitioners do various types of dentistry, and thus the quality of the dental work may be established. For instance, a mouth that had been entirely reconstructed with gold inlays and bridges with beautifully fitted margins would place the subject in a comparatively high income group. Well fitted inlays and poorly fitted inlays or poorly formed amalgams in the same mouth might indicate that more than one dentist had worked on that particular mouth. Poor dental work and good dental work in the same mouth might indicate a change of economic level in the lifetime of the person; certainly, a change of dentists would be indicated. These factors might be helpful in establishing identity.

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3. Armstrong, W. D., and Brekhus, P. J.: J. Dent. Research **17**:27, 1938.

Gustafson [4] recently demonstrated another important method of identification. He has shown that by making a careful microscopic examination of ground sections of teeth under polarized light it is possible to determine accurately which teeth of a given group of teeth came from the same person. This is done by comparing Ebner's lines in the dentin on one tooth with corresponding Ebner's lines in another tooth. If subsequent to an explosion or some other mutilating accident, only a number of teeth remained, a method such as this would prove invaluable in determining whether the teeth belonged to one or more than one person; in fact, the exact number of persons involved could be accurately determined.

In the recognition of injury, several factors may be of importance. A case is called to mind in which no marks of violence were found anywhere on the skeletal remains except in the jaws and the teeth. Careful examination of the jaws and the teeth showed that the enamel of various cusps of opposing teeth had been fractured. Besides the fractured enamel of these cusps, it was discovered that the left side of the lower jaw was fractured just below the head of the condyle. By assembling the jaws in their proper positions and by noting that buccal cusps of the upper jaw and lingual cusps of the lower jaw on the left side were fractured, it became quite apparent that a hard blow must have been delivered to the right side of the lower jaw in order for those particular cusps to be fractured in those particular places as well as for the mandible to be fractured.

In the case described there was no soft tissue to aid in the examination. When soft tissue is present, additional information may be obtained. A blow delivered to the jaw before death would in all probability loosen a tooth or teeth. This loosening would be accompanied with hemorrhage and compression of the supporting bone of the tooth socket. Blood cells present around the neck of the tooth or blood cells infiltrating into adjoining tissue spaces, or both, would indicate antemortem loosening. If extracellular blood could not be demonstrated, the presence of foreign body cells, phagocytic in type, might be demonstrated in tissue spaces where hemorrhage had been. If a tooth had been loosened post mortem, in transferring the body from one place to another, neither extracellular blood nor foreign body cells could, of course, be demonstrated.

Likewise, antemortem loss of a tooth may be differentiated from postmortem loss. If a tooth is lost ante mortem, hemorrhage occurs and a clot forms immediately (except in rare instances). A clot in the socket with organization taking place by fibroblastic proliferation would indicate healing and thus antemortem loss. Postmortem loss would show, of course, no hemorrhage, no organization and no attempt

4. Gustafson, G.: J. Am. Dent. A. **35**:720, 1947.

toward healing, with sharp, jagged edges of supporting bone remaining. This is entirely in opposition to nature's method of producing a smoothly rounded alveolus with resorption of sharp bony spicules following an extraction.

Differentiation of a tooth lost by extraction and a tooth lost by avulsion, or a knocking out by means of a blow, could also be demonstrated. Generally speaking, a tooth extracted by an oral surgeon or a dentist would show some evidence of surgical procedure, i. e., flap formation to facilitate removal of the tooth or root, presence of bone grindings produced by a surgical burr, filed edges of a bony socket or cleancut areas of bone produced with a rongeur to produce a uniform smooth result. Avulsion, on the other hand, in all probability would present a rather crude picture. Even if the tooth were completely knocked out of the socket, the jagged edges of remaining bone, splinters of buccal or lingual plate, areas of compressed bone or fractures of roots or crowns of adjacent remaining teeth would, in all probability, exist. It would be practically impossible to knock out completely one tooth in an arch without showing some evidence of fracture, or loosening of teeth adjacent to it. This could best be demonstrated by a roentgenogram of the area along with a careful examination.

Within limits, it is possible to estimate the time that a tooth was extracted before death. If a tooth was extracted one year before death, a roentgenogram of the area would show the socket completely filled with new bone, the soft tissue, of course, being completely healed. A six month antemortem extraction would be indicated by soft tissue that had completely healed and a roentgenogram showing the socket filled with new bone but with the outline of the location of the roots still present. The picture would be similar for a two or three month antemortem extraction, with less bone having filled in the socket. A one or two day antemortem extraction would be shown by a clot with definite organization begun. A one week antemortem extraction would be shown by a definite healing and filling of the socket with organized clot. A two or three week antemortem extraction without complications would be indicated by soft tissue that had completely healed but a socket not yet filled with bone. This would have to be demonstrated, of course, by roentgenogram.

In regard to the relative degrees to which teeth and bones may be destroyed by heat, Schirnding [5] observed:

> In the great fire of the . . . Opera-Comique . . . in Paris . . . the effects of the high temperature . . . [showed]that . . . in most cases the teeth were better preserved than other parts of the body on account of their sheltered position. The materials used in the fillings withstood the influence of the fire to various extents, according to the grade of their composition. The teeth them-

5. Schirnding, H.: Dent. Cosmos **76**:853, 1934.

selves were burned in the most varied ways. The most damaged were always found in skulls that had been more or less completely destroyed by the flames. In such cases the teeth were reduced to small stumps, whitened by the fire, were easily loosened from their alveoli, or had already fallen out. The charred cementum, with remains of calcinated enamel sticking to it, was also found with the root still sticking in the alveolus.

Schirnding stated further:

The changes to which the teeth are subject through the influences of high temperature are of great importance in forensic medicine. The darker coloring of those teeth [meaning those teeth subjected to great heat] that have previously been the most healthy may easily be mistaken for caries or injuries caused by some occupation, while, on the other hand, teeth that had previously been the darker ones may become lighter or even white in the process of calcination. This is especially observable in teeth that have been heavily coated with tartar. Of course, these alterations considerably increase the difficulty of making a diagnosis. In the same way, where pieces of the teeth have been broken off during or after their exposure to the influence of great heat, bodily injuries may be supposed to have been the cause, and the difficulty of identifying a person is thus increased.

The importance of establishing personal identity is evident. Continued effort on the part of members of the dental profession to record and to make available accurate examinations of the mouths of patients plus close cooperation between the dentist and the medical examiner would do much to aid in the establishing of identity by means of the teeth and their supporting structures.

CONTROL OF HEPATIC COCCIDIOSIS OF RABBITS WITH SUCCINYLSULFATHIAZOLE U. S. P.

A Study of the Mode of Action of the Sulfonamides

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THERE have been few investigations concerning sulfonamide prophylaxis or therapy of protozoan diseases. The protozoa probably pursue their first life cycle in the intestinal lumen; from there the sporozoites pass to the liver through the radicles of the vena portae and reach Kiernan's spaces, whence they make their way into the bile ducts.

It was within the scope of the present investigation to ascertain whether succinylsulfathiazole U. S. P. ("sulfasuxidine" N. N. R.) can prevent the coccidia from reaching the liver or from proliferating within the intestinal lumen. Since sulfonamides may inhibit cell division or motility, it was surmised that sporulation of the parasites and invasion of new biliary duct cells might be prevented or delayed by the administration of the drug.

EXPERIMENTAL PROCEDURE

As a preliminary step, the rabbits were given succinylsulfathiazole to eliminate, if possible, any intestinal coccidia which they might harbor. The rabbits were then divided into three groups of 7 each.

Group 1. The animals received no treatment.

Group 2. Each of the animals received one single dose of 10,000 oocysts by mouth.

Group 3. Each of the animals received one single dose of 10,000 oocysts by mouth and a daily dose of 0.5 Gm. of succinylsulfathiazole mixed with the feed.

The animals were killed at the eighteenth day following infection and their organs examined. The first group, as was to be expected, showed no lesions of any sort. The animals of the second group had enlarged livers, studded with numerous small grayish nodules all through the parenchyma, and a biliary tree uniformly dilated.

The animals of the third group showed a smooth liver with no lesions of any kind visible to the naked eye. The appearance of the organ was similar to that in the first group with the exception of moderate engorgement and a slight increase in size. As the findings were identical in all the animals of the succinylsulfathiazole series, it was assumed that the sulfonamide had prevented the infection.

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The oocysts which were collected aseptically from the gallbladders of the infected animals were immersed in four different solutions: a potassium dichromate solution, an aqueous solution of sulfathiazole (1:2,000), an aqueous solution of succinylsulfathiazole (1:700) and an 0.85 per cent sodium chloride solution.

Neither sulfathiazole nor succinylsulfathiazole in the concentrations noted here prevented the sporulation of the oocysts.

PATHOLOGIC OBSERVATIONS

The histologic study of the livers was made by means of paraffin sections stained with hematoxylin and eosin.

Group 1.—The liver showed normal structure with the hepatic trabeculae symmetrically arranged around the centrolobular vein and the sinusoids lined by flat endothelial cells. The spaces of Kiernan showed small slitlike biliary lumens lined by cylindric epithelial cells in one single row and a normal framework of fibroblasts and collagen fibers. The liver cells occasionally appeared granular with small vacuoles outside the cytoplasm; however, no pigment granules or other inclusions were present.

Group 2.—Under low power magnification were seen enormously dilated biliary ducts with their profusely proliferating epithelium thrown into countless papillary folds. Either free in the lumen or parasitizing epithelial cells, numerous gametocytes of Eimeria stiedae were seen, and occasionally also schizonts in process of division or free sporozoites. The cellular reaction around the ducts was moderate and included chiefly macrophages, plasma cells and fibroblasts; it sometimes extended to the neighboring areas of the liver, where it penetrated into the hepatic lobules. Sometimes inside the hepatic lobules isletlike areas were seen, in which newly formed biliary capillaries seemed to originate from the liver cells themselves. The liver cells were well preserved, and, except for the infiltration which distored the normal structure, showed no signs of pathologic disturbance.

Group 3.—The structure of the liver was not greatly distorted; the Kiernan spaces were of a normal appearance except for a marked cellular reaction, which sometimes involved also the peripheral area of whole lobules. The biliary ducts were small and slitlike or round, and were lined by single rows of cylindric epithelial cells. Around the ducts and occasionally even between the cells lining the lumens were numerous macrophages, lymphocytes and fibroblasts. No parasites were found even where the cellular reaction was most pronounced. The liver cells were swollen and showed granular, vacuolated cytoplasm; their nuclei, however, were preserved and appeared normal. As the cells of untreated infected animals did not show any similar lesion, it is probable that the cytoplasmic changes were due to the administration of succinylsulfathiazole rather than to any toxic products of the parasites.

COMMENT

Most of the published results of experiments with sulfonamides carried out on protozoa deal with malaria of birds and monkeys because of the close connections with human malaria [1] in certain aspects.

1. Coggeshall, L. T.: Proc. Soc. Exper. Biol. & Med. **38**:768, 1938; J. Bact. **39**:30, 1940; J. Exper. Med. **71**:13, 1940.

Succinylsulfathiazole has not been used experimentally to date on coccidioses; thus, so far as my knowledge extends, this is the first report of successful prophylaxis of coccidiosis of the liver by administration of this drug. The danger of hepatic damage seems slight. Lindelof [2] stated that sulfonamides cause an increase of tonus and an "acceleration of pendulum" of the living intestine; Climenko, McChesney and Messer,[3] who studied the effects of continued administration of sulfathiazole in dogs, found that it may influence renal excretion but that it does not impair hepatic function.

How does succinylsulfathiazole act on the parasite? Chodat and Olivet [4] found that sulfanilamide interfered with the sporulating activity of algae, and Lwoff, Nitti, Trefouel and Hamon [5] noted that cell division of the flagellate Polytomelia caeca is inhibited by this same compound. Thomas was the first to observe that sulfanilamide inhibits division of eggs of the sea urchin, and Fisher, Henry and Low [6] compared its action to that of typical narcotics. While further work on the action of succinylsulfathiazole on coccidia is being carried out in the present investigation, there is reason to believe that perhaps the mechanism of cell division or of sporulation and excystation of the parasites is impaired by the sulfonamide.

The cellular reaction encountered in the livers of animals treated with succinylsulfathiazole might be interpreted superficially as due to stimulation of immunizing mechanisms by the drug. However, the effect reasonably could represent a natural reaction of the organism to an infection of decreased intensity and thus could be indirectly related to the action of the drug itself.

This reaction which has been observed indicates that the sporozoite must have reached the liver, where either their motility was impaired or their growth and division were inhibited. As the biliary epithelium showed no deviation from the normal and no attempt at proliferation, it must be assumed that progress from the portal vein to the biliary duct had been impeded and that a cellular reaction had taken place to eliminate the parasites. Both mechanisms may be postulated for the drug action: inhibition of motility and inhibition of cell divisions, each leading to the elimination of the parasites and to the establishment of a strong cellular reaction.

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2. Lindelof, S. A.: Chem. Abstr. **37**:4466, 1943.

3. Climenko, D. R.; McChesney, G. W., and Messer, F.: Proc. Soc. Exper. Biol. & Med. **46**:124, 1941.

4. Chodat, F., and Olivet, R.: Arch. sc. phys. nat. **22** (supp.):143, 1940; cited by Henry, R. J.: The Mode of Action of Sulfonamides, New York, Josiah J. Macy Jr., 1944.

5. Lwoff, A.; Nitti, F.; Trefouel, J., and Hamon, V.: Ann. Inst. Pasteur **67**:9, 1941.

6. Fisher, K. C.; Henry, R. J., and Low, E.: J. Gen. Physiol. **27**:469, 1944.

Some authors [7] have advanced the idea that sulfonamides may constitute a factor in the enhancement of the mechanism of immunity. As toxins are not affected by contact with sulfonamides, the increase of resistance seems only an incidental factor in the action of the drugs. The principal action is exercised on the micro-organisms themselves.

Sometimes a certain result may be due to a composite of inhibitive effects without the inhibitors having any mutual interrelationship. The simultaneous administration of a sulfonamide drug and serum, for example, may result in a more prompt recovery than administering of either one by itself, but this synergistic effect does not mean that the action of one has served to enhance the effect of the other. We can assume, instead, an independent action of each on the bacteria and a cumulative effect in stimulating or reawakening the defensive processes of the organism. Further speculation on this subject needs additional investigation.

SUMMARY

Succinylsulfathiazole has been tested on rabbits infected with Eimeria stiedae. In the first series of experiments, the rabbits were infected with 10,000 oocysts of E. stiedae, and one half of them were given 0.5 Gm. of the sulfonamide, mixed with the feed, daily for a period of 14 to 16 days. The untreated infected animals showed typical lesions of coccidiosis of the liver, whereas the infected animals treated with succinylsulfathiazole showed no lesions of the liver. Since the results were uniform in all the animals and no toxic effects were observed as a consequence of the administration of the drug, it is hoped that this widespread infection may be successfully treated. From the study of the histologic material it seems likely that the sporozoites are carried through the portal vein to the liver, where either they are inhibited in their progression toward the biliary epithelium or, if they reach there, they are prevented from dividing.

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7. Findlay, G. M.: Lancet **2**:761, 1941. Lyons, C., and Mangiaracine, A.: Ann. Surg. **108**:813, 1938.

SIGNIFICANCE OF AGONAL CHANGES IN THE HUMAN LIVER

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DESPITE great efforts of clinicians and pathologists, the correlation of morphologic changes of the liver and clinical manifestations remains problematic. In many instances the functional significance of alterations seen under the microscope cannot be evaluated. This difficulty is met in the experimental animal when marked histologic changes are found in the absence of jaundice or definite impairment of hepatic function. In regard to the human subject the problem is augmented if the histologic studies are based on autopsy material. The occurrence of marked premortal, agonal and postmortal changes of the liver is brought home by the study of specimens obtained for biopsy; the use of such specimens has been rather extensive in recent years.[1] This problem invites a systematic investigation of the hepatic changes that occur in the agonal period and an evaluation of their significance.

To study this problem, three different approaches were used: 1. The histologic picture as seen in autopsy material was compared with that seen in biopsy specimens without any attempt to study the same liver in premortal and postmortem material. 2. In the rare cases in which a liver specimen could be obtained at autopsy a short time after a biopsy had been made, premortal and postmortal histologic appearances were compared. 3. Livers of healthy persons who died instantaneously, as in crashes, were compared with those of persons who died suddenly but with an interval of more than 10 minutes between the onset of the injury or the disease and the actual death. In cases of the latter type, therefore, an agonal period of more than 10 minutes has to be assumed.

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1. (*a*) Iversen, P., and Roholm, K.: Acta med. Scandinav. **102**:119, 1939. (*b*) Dible, J. H.; McMichael, J., and Sherlock, S. P. V.: Lancet **2**:402, 1943. (*c*) Mallory, T. B.: J. A. M. A. **134**:655, 1947. (*d*) Jones, M. C., and Volwiler, W.: M. Clin. North America **31**:1059, 1947. (*e*) Popper, H.; Steigmann, F.; Meyer, K. A.; Kozoll, D. D., and Franklin, M.: Am. J. Med., to be published.

There are in the literature ample references to postmortal autolytic processes, which are especially rapid in cases of so-called acute atrophy of the liver. This was pointed out by Umber,[2] Versé[3] and Hanser[4] many years ago, after comparing a biopsy specimen taken during a surgical operation with autopsy material obtained shortly thereafter. In their studies emphasis was placed on the changes of the cells of the hepatic parenchyma. In my own experience,[5] central necroses, which were absent in biopsy specimens obtained shortly before death, were observed at autopsy. That the postmortal autolytic processes are enhanced in cases of infection or toxemia is well known to every pathologist. Glycogen present in liver cells as indicated by histochemical methods or the characteristic granulation of the cytoplasm has been considered as evidence of sudden death,[6] and medicolegal significance has even been ascribed to it.[7]

MATERIAL AND METHOD

For the general comparison of autopsy and biopsy material 226 specimens obtained by needle aspiration (performed by D. D. Kozoll[10]) from 156 patients and 108 specimens obtained by surgical excision during laparotomy (performed by K. A. Meyer[8]) were available. The great majority of these specimens revealed definite pathologic changes; in the surgical series, however, there were some fairly normal pictures, as, for example, in specimens from patients with peptic ulcer. Some of the biopsy specimens were fixed in formaldehyde solution U. S. P. diluted 1:10, formaldehyde-Zenker or acetic acid–Zenker solution; the majority of them were hardened in Carnoy solution, which is optimal for the study of the perisinusoidal spaces.[9] Autopsy material fixed in different types of solutions was used for comparison.

Biopsy and autopsy materials of the same patient were available in 38 cases. In only 4 instances was the interval of time between autopsy and biopsy shorter than 48 hours, and these cases were utilized for comparison.

The third part of the study was based on material observed in two histopathologic centers of the Army; it includes 351 subjects, all males of military age; instances in which the subject was over 45 years of age were excluded. These soldiers were healthy until shortly before death and died suddenly—some from causes connected with military training and the rest from diseases or accidents not directly connected with military life. Death had occurred within

2. Umber, F.: Berl. klin. Wchnschr. **57**:125, 1920.
3. Versé: Berl. klin. Wchnschr. **57**:127, 1920.
4. Hanser, R.: Verhandl. d. deutsch. path. Gesellsch. **18**:263, 1921.
5. Popper, H.: Wien. klin. Wchnschr. **49**:207, 1936.
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8. Meyer, K. A.; Steigmann, F.; Popper, H., and Walters, W. H.: Arch. Surg. **47**:26, 1943.
9. Eppinger, K.; Kaunitz, H., and Popper, H.: Die seröse Entzündung. Berlin, Julius Springer, 1935.

24 hours after the accident or the onset of disease. Cases in which the history revealed that symptoms of illness had been noted a longer time before death were excluded. On the basis of the information available in the history, these 351 cases of sudden death were divided into two groups: 96 instances of instantaneous death, the result of a trauma, and 255 cases in which death occurred between 10 minutes and 24 hours after the onset of a disease or the time of an accident. For comparison, sections of the livers of 160 soldiers who died of a sequela of an accident or of a disease more than 24 hours after the accident or the onset of the disease were studied.

In addition to paraffin sections stained with hematoxylin and eosin, others stained with Mallory's aniline blue stain or his modification of the Masson stain and Gömöri's or Foot's reticulum fiber stain were studied. In selected instances, slides stained for glycogen with the periodic acid method of McManus[10] were examined.

RESULTS

Comparison of the Premortal and Postmortal Histologic Appearances of the Liver Based on Biopsy and Autopsy Material in General.—As repeatedly emphasized, the cytoplasm of the liver cells in hematoxylin-eosin sections of biopsy specimens was lightly stained. In the absence of marked pathologic changes it revealed a uniform, fine vacuolation and granulation. These were due to the removal of cytoplasmic glycogen which occurs in the embedding process. If the biopsy specimen was taken shortly after a therapeutic intravenous injection of dextrose, the cells appeared swollen. In autopsy material the cytoplasm was usually darker except for the presence of fat vacuoles. The cells appeared similar to those of biopsy specimens only in instances of acute death or after extensive premortal dextrose therapy. In biopsy specimens the space between liver cell cords and sinusoids was, as a rule, completely obliterated. The larger and thicker reticulum fibers which were parallel to the axis of the liver cell cords were well impregnated in silver preparations and stained with aniline blue. However, the cross fibers were not visualized in aniline blue stains, since the sinusoidal wall and the Kupffer cells appeared attached to the liver cell cords. Only in the presence of edema, which was primarily observed in toxic hepatitis, in long-standing biliary obstruction (biliary hepatitis) and especially in the central portion of the lobule in congestion, was the perisinusoidal space seen. It was almost invariably visible in autopsy specimens. It varied in width, and in a number of cases, considered instances of hepatic edema, marked albuminoid debris was seen in it; the sinusoids appeared compressed in the instances in which edema was fully developed. The sinusoidal walls with the Kupffer cells were detached from the liver cells almost without exception. The perisinusoidal space was traversed by a large number of fine cross reticulum fibers, which connected in the form of an arch and occasionally interlaced the thicker fibers adherent to the perisinusoidal wall with the liver cell cords. They appeared more in number and better impregnated than those in biopsy specimens.

Dissociation of individual liver cells from the cell cords was occasionally observed in biopsy specimens. These cells appeared round; their cytoplasm was eosinophilic. A communication between the bile capillary in the center of the cords and the perisinusoidal space could hardly be visualized, despite the dissociation. Denudation of the framework in the center of the lobule with development of central necrosis was hardly ever visible, even when the cytoplasm of the liver

10. McManus, J. F. A.: Nature, London **158**:202, 1946.

cells in the central portion had lost its basophilia or when definite damage of liver cells could be established by morphologic or functional findings. In autopsy specimens, however, disruption of the liver cell cords with dissociation of the cells was not uncommon, even in instances in which the damage of liver cells was not severe. The dissociated cells might reveal normal basophilia of the cytoplasm or intact nuclear staining. A funnel-shaped communication between the lumen of the bile capillary and the perisinusoidal space was occasionally demonstrable. This was especially clear if the bile capillaries were dilated in the presence of jaundice.

As to the examined experimental animals, which included mice, rats, rabbits, dogs and guinea pigs, the perisinusoidal spaces were hardly ever visible even in postmortem material; the cross fibers of the reticulum network were less clearly demonstrated than in the liver of the human subject. As far as the perisinusoidal

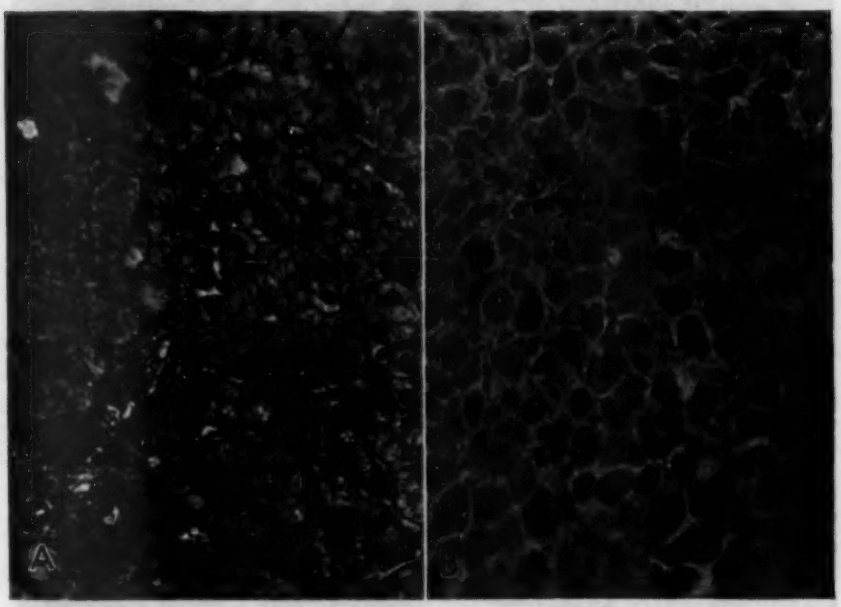

Fig. 1.—*A,* biopsy specimen of liver taken 13 hours before death in a case of mild toxic hepatitis. The structure of the liver cell cords is only slightly distorted, and the perisinusoidal spaces are almost completely closed. The liver cells appear to be rich in glycogen.

B, autopsy specimen obtained in the same case as *A*. There is marked dissociation of the liver cell cords, and the tissue spaces between them and the sinusoids are open. The isolated liver cells appear poor in glycogen.

space is concerned, the liver of the examined animals resembled human liver as seen in biopsy specimens.

Comparison of the Premortal and Postmortal Histologic Appearances of the Liver Based on Biopsy and Autopsy Specimens of the Same Liver.—Differences between biopsy and autopsy specimens the same as those described in the foregoing section of this paper could be demonstrated in the 4 cases available for this part of the study. Of special value is a case of a 66 year old Negro woman with a mild degree of toxic hepatitis without established exposure in the history. Only slight jaundice was present; the liver appeared enlarged and

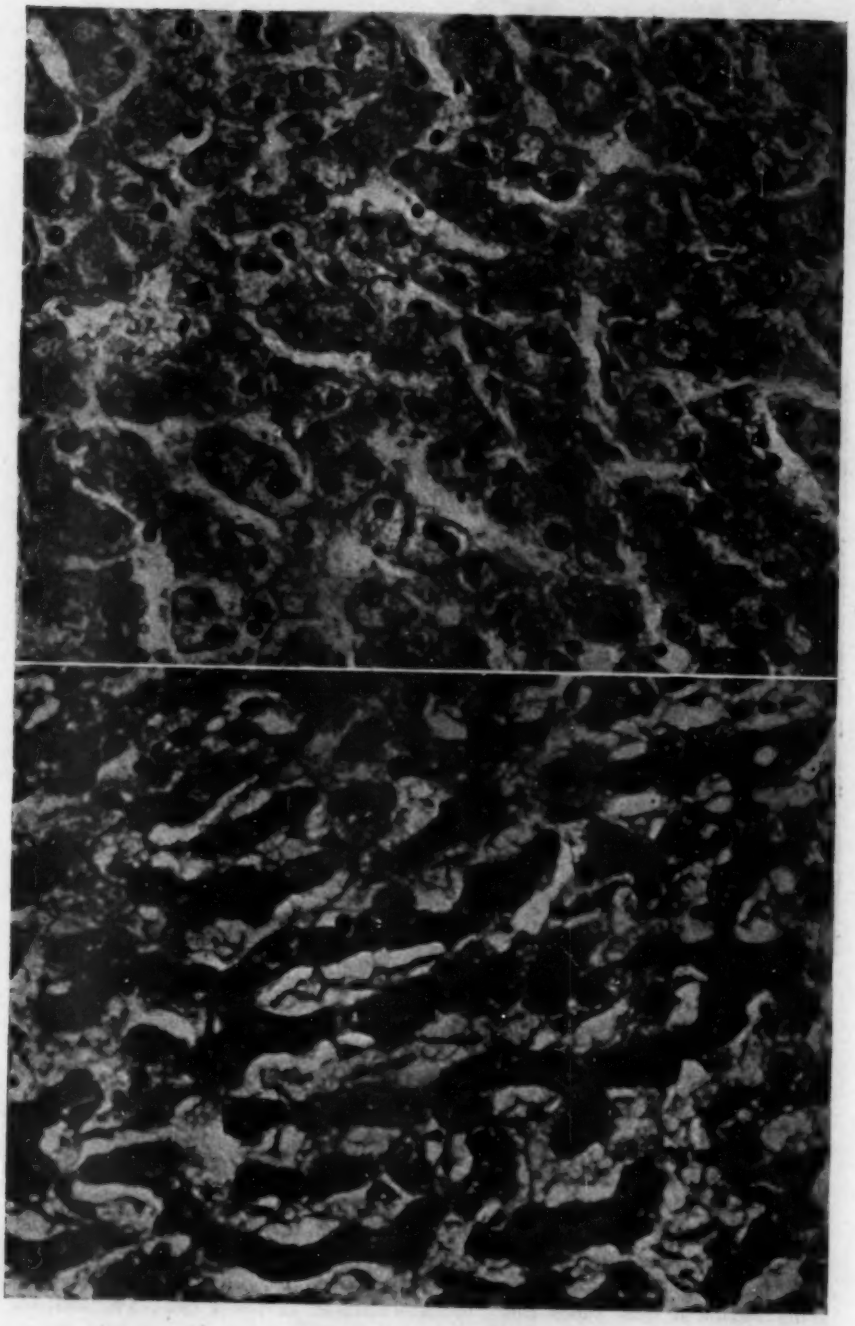

Figure 2

(See legend on opposite page)

tender and had a sharp edge. The spleen was easily palpable. Five hours after aspiration biopsy, signs of shock with anemia developed, and the patient died within thirteen hours after the biopsy. At the autopsy, performed seven hours after death, the abdomen contained large amounts of liquid and clotted blood; the total amount was estimated at 2,500 cc. Three small perforations on the surface of the reddish brown liver led into a 5 cm. long channel, at the end of which a laceration of a large tributary of the portal vein was found. Histologically (fig. 1 *A*), the biopsy specimen revealed a well preserved lobular pattern; however, the arrangement of the liver cell cords was somewhat irregular. The liver cells varied in size; the cytoplasm was finely granular, rich in glycogen, rarely clumped, and occasionally some bile pigment granules or medium-sized fat droplets were seen. The nuclei varied markedly in size, and some were ballooned. Nowhere were liver cells isolated. The bile capillaries were narrow, and communications between them and the perisinusoidal space were not made out. The latter was in most places obliterated and was represented by a small slit containing some albuminoid material only in the center of the lobule. The

Relation of the Width of the Perisinusoidal Space to the Duration of the Agonal Period and the Cause of Death

Cause of Death	Instantaneous Death, Cases in Which Perisinusoidal Spaces Were			Sudden Death, Cases in Which Perisinusoidal Spaces Were		
	Obliterated	Partially Open	Open	Obliterated	Partially Open	Open
Crash or blast	26	1	1	1	1	8
Injuries of the brain	32	5	7	3	6	54
Cardiac tamponade and hemorrhage	21	1	1	2	9	22
Heart failure	0	0	0	3	1	54
Suffocation	0	0	0	2	0	32
Strangulation	0	0	0	1	8	10
Drowning	0	0	0	0	0	38
Total	79	8	9	12	25	218

sinusoids contained a moderate amount of erythrocytes and also some white cells. Infiltration with round and few polymorphonuclear cells was found in the portal triads. In the autopsy specimen (fig. 1 *B*) the glycogen content of the liver cells was reduced and their eosinophilia increased in comparison with the biopsy specimen; the nuclei appeared not changed. The liver cell cords revealed marked dissociation; the rounded individual cells were in most places separated from each other. Consequently, in many places the bile capillaries appeared ruptured, and some bile seemed to leak through funnel-shaped communications from the bile capillaries into the perisinusoidal spaces. The latter were markedly widened and filled with albuminoid debris. Some sickled erythrocytes were noted in the larger vessels, but rarely in the sinusoids.

Fig. 2.—Hematoxylin-eosin stain. *A*, liver of a soldier dying instantaneously in a crash (Army Institute of Pathology negative 93993). The lining of the sinusoids is adherent to the liver cell cords, and no perisinusoidal space is visible.

B, liver of a soldier dying from an injury of the head more than one hour after the accident (Army Institute of Pathology negative 93964). The perisinusoidal spaces are wide and filled with albuminoid debris.

Figure 3

(See legend on opposite page)

Similar differences between autopsy and biopsy specimens were apparent in the 3 other cases of this series; however, more outspoken pathologic changes somewhat obscured the picture.

Influence of the Duration of the Agonal Period on the Histologic Appearance of the Liver.—Some minor alterations from the norm were found in the studied cases of soldiers dying suddenly, such as irregularly scattered fat droplets or wear and tear pigment in the form of branched coarse brown granules. The main emphasis, however, was laid in this study on the relation between the wall of the sinusoids and the liver cell cords. In one group (fig. 2*A*), the sinusoidal lining was closely attached to the liver cell cords, and no perisinusoidal spaces were visible. The wall itself appeared rather thin in sections stained with hematoxylin and eosin and as a fine blue line in sections stained with aniline blue and the Masson stain. The reticulum fibers were represented by one solid string adherent to the wall of the sinusoids (fig. 3*A*). Cross fibers were impregnated only in places. They were best seen in the vicinity of the portal and central fields, where they connected with dense collagenous connective tissue. In another group of livers, with hematoxylin-eosin stain a cleft (fig. 2*B*) containing albuminoid debris was visible between liver cell cords and perisinusoidal lining. It varied but was more noticeable in the central part of the lobule. The Kupffer cells, though separated from the liver cell cords, were not active. The sinusoidal wall was indicated in connective tissue stains by a distinct blue strand, clearly separated from the liver cell cords. Some fine fibers seemed to traverse the perisinusoidal space, and the connective tissue framework was better stained than in the first group. In silver impregnations the long fibers parallel to the axis of the liver cell cords appeared split into several thin fibers with fine clefts between them. A dense net of arcuated or interlaced cross fibers extended from the capillary walls to the liver cell cords. As a whole, more of the reticulum framework was impregnated, owing to its expansion. Between those two groups transition occurred, and consequently a third group was established in which the perisinusoidal spaces were in places open, with expanded reticulum framework, and in others absent.

The perisinusoidal spaces were obliterated in the great majority of the cases of instantaneous death, in 82.3 per cent, and only in a small number were they partially (8.3 per cent) or completely open (9.4 per cent) (table). On the other hand, the perisinusoidal spaces were open throughout in the great majority of cases (83.5 per cent) in which death was sudden but the agonal period of longer duration than 10 minutes, and only in a small number (5.3 per cent) obliterated.

The greatest number of exceptions to this rule occurred in injuries of the central nervous system, which included crash or gunshot trauma of the brain. It was rather difficult to ascertain how quickly death developed in these instances. Death was delayed in a relatively large number of them; it was in this group

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Fig. 3.—Silver impregnation of reticulum fibers. *A*, liver of a soldier dying instantaneously in a crash (Army Institute of Pathology negative 93948). The reticulum framework is represented by stringlike axial fibers, whereas cross fibers are hardly recognized.

B, liver with severe hepatic edema from a patient with nephrosis (Army Institute of Pathology negative 93947). The expanded reticulum framework is represented by split axial fibers and many cross fibers extending through the wide perisinusoidal space.

that fairly often the perisinusoidal spaces were only partially opened. Far more uniform were the findings in instances in which a sudden crash, the result of an airplane accident or a grenade explosion, involved the greater part of the body. In such instances, when the patient survived for a while, the spaces were usually open. The tissue spaces were also uniformly obliterated in instantaneous death the result of rapid exsanguination produced by laceration of the heart or a large vessel due to a gunshot or a stab wound. When an extensive hemorrhage caused hemothorax or hemoperitoneum and death was sudden but delayed for more than 10 minutes or even up to 1 hour, the tissue spaces were completely or at least partially open.

Under heart failure were listed instances of coronary thrombosis, interstitial myocarditis without known preceding symptoms, dissecting aneurysm and the like. In some cases of sudden cardiac failure the autopsy failed to reveal the actual cause. The tissue spaces were very wide, especially in the central portion of the lobule, in almost all cases of this group in which circulation stopped at least 10 minutes after the onset of the heart failure. Evidence of acute passive congestion could sometimes be seen. Instances of subacute or chronic congestion were eliminated. Open and occasionally wide tissue spaces were observed in cases of strangulation, despite the rather sudden death. The same was found in a group under the heading of suffocation, in which death was due to pressure placed on the thorax in an accident, to illuminating gas intoxication mostly on a suicidal basis, to cyanide and strychnine intoxication, to anaphylaxis, to lightning, to electrocution and, in a number of cases, to heat stroke. The open and rather wide tissue spaces were filled with granular albuminoid material. The perisinusoidal spaces were at least partially open and often rather wide also in cases of drowning. In some cases of the latter, signs of decomposition were noted, but the spaces were wide even with intact protoplasmic and nuclear staining.

In 13 of the cases just described, alcoholic intoxication contributed to the cause of the fatal accident but did not influence the relation between the speed of death and the width of the tissue spaces. They were closed in 2 instances of instantaneous death and open in the remaining cases.

Little information as to the cytoplasmic structure could be obtained from this material, because the fixation was not uniform. Glycogen stains were not studied; if a fine, regular granulation or vacuolation of the cytoplasm of the liver cells may be considered an indication of the presence of glycogen and the dark homogeneous cytoplasm of the narrower liver cells an indication of its absence, one finds closed tissue spaces, as characteristic of instantaneous death, usually associated with high glycogen content. Partially or fully open tissue spaces as seen in sudden, but not instantaneous, death were observed with both high and low glycogen content. This indicates that open tissue spaces may occur in the presence of swollen liver cells.

In the livers of 160 soldiers dying longer than 24 hours after the onset of their fatal illness, the perisinusoidal spaces were wide open except for 2 instances in which there was diffuse fatty metamorphosis. The width of the perisinusoidal spaces and the deposition of albuminoid material depended on the underlying disease. The distribution and extension of the hepatic edema found in different diseases confirmed the observations of Keschner and Klemperer.[11] Marked widening of the perisinusoidal spaces was usually associated with thickening of

11. Keschner, H. W., and Klemperer, P.: Arch. Path. **25**:583, 1936.

the lining of the sinusoids in hematoxylin-eosin sections; the wall revealed a double contour. With fiber stains the widening appeared to be caused by a pushing apart of the axial reticulum fibers, between which fine clefts were visible. With silver preparations the reticulum network was especially clear in instances of hepatic edema (fig. 3 *B*).

COMMENT

The presented observations reveal that marked alterations occur in the liver during the agonal period which should be taken into consideration in correlating clinical with histologic observations. The comparing of autopsy and biopsy material as such does not permit differentiation between agonal and postmortal changes. This is better accomplished by studying autopsy material after various durations of the agonal period. The most conspicuous cytoplasmic changes occurring in the agonal period are the well known disappearance of the cytoplasmic glycogen and the subsequent darkening of the cytoplasm in hematoxylin-eosin sections. Other cytoplasmic changes may depend on nutrition and other physiologic factors about which in the cases studied no information was available. Less well appreciated is the fact that dissociation of the liver cell cords with isolation of individual liver cells may be the result of agonal or of postmortal processes. Such changes occurring in autopsy material should only with great caution be correlated with any intravital process. I myself have been guilty of omitting this caution.[12] The point is important in the explanation of the genesis of jaundice. Eppinger[13] has claimed that regurgitation jaundice the result of damage of liver cells or of biliary obstruction is due to rupture of the bile capillaries in the centers of the liver cell cords with formation of funnel-shaped communications between them and the perisinusoidal spaces. In biliary obstruction the dilated ramifications of the bile capillaries between the liver cells approach the perisinusoidal space and are supposed to rupture into it. The parenchymatous type of jaundice was explained by communications between bile capillary and tissue space produced by a break-up of the liver cell cords. Studies of biopsy specimens, however, fail to support Eppinger's intriguing explanation of regurgitation jaundice, since the assumed communication is not found, as pointed out by Roholm and Iversen.[14] The present study, also, does not favor this explanation, since the dissociation, which forms the basis of this theory, may represent an agonal or a postmortal process. The contention that regurgitation jaundice is associated with

12. Kirshbaum, J. D., and Popper, H.: Arch. Int. Med. **65**: 465, 1940. Steigmann, F.; Popper, H., and Meyer, K. A.: J. A. M. A. **122**:279, 1943.

13. Eppinger, H.: Die Leberkrankheiten, Berlin, Julius Springer, 1937.

14. Roholm, K., and Iversen, P.: Acta path. et microbiol. Scandinav. **16**: 427, 1939.

changes in the smallest bile ducts appears to be more justifiable than the belief that it is associated with a hepatocellular process, as recently emphasized by Watson and Hoffbauer.[15]

The fact that central necroses are rarely observed in biopsy material despite their common occurrence in autopsy specimens of similar nature may suggest that they, too, may develop in the agonal period. However, more extensive observations are necessary to confirm this hypothesis.

Significant and so far apparently not appreciated changes of the connective tissue framework and the perisinusoidal spaces occur in the agonal period. The spaces seem, as a rule, to be closed during life, and hepatic edema is a rare occurrence on the basis of this observation. It is common in autopsy material. It develops within a few minutes, as judged from a comparison of the livers of previously healthy soldiers who died instantaneously and those of others who died after a short agonal period. Dilatation of the perisinusoidal spaces of the liver, which is usually associated with accumulation of albuminoid material, has been considered as a morphologic sign of damage of the sinusoids. As a result, protein normally retained within the capillary bed escapes into the perisinusoidal space and binds water. Protein passes through the capillary wall more readily in the liver than in other organs. Roessle [16] considered the escape of protein as inflammatory in nature and spoke of serous hepatitis. He expressed the belief that the edema fluid may elicit connective tissue formation and thus considered the phenomenon as a potential initial stage of cirrhosis of the liver. Subsequently, this concept was elaborated on, and serous inflammation in general was considered an important basic phenomenon.[9] Serous hepatitis was supposed to be the morphologic substrate of early damage of liver cells and an important feature of parenchymatous hepatitis. Bloom and Maximow,[17] however, denied the existence of these spaces and emphasized that the lymphatic channels reach only into the portal and central fields. Keschner and Klemperer,[11] investigating the significance of the widening of the perisinusoidal spaces, assumed two factors: (1) a hydromechanic one (mechanical edema) due to circulatory failure and (2) increased permeability of the sinusoidal wall (primary edema) occurring in various morbid conditions. These authors considered the term "serous hepatitis" as inappropriate.

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15. Watson, C. J., and Hoffbauer, F. W.: Ann. Int. Med. **25**:196, 1946.

16. Roessle, R.: Entzündungen der Leber, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie and Histologie, Berlin, Julius Springer, 1930, vol. 5, pt. 1.

17. Bloom, W., and Maximow, A. A.: A Textbook of Histology, ed. 4, Philadelphia, W. B. Saunders, 1944.

According to the studies presented here, a short agonal period suffices to produce a significant degree of hepatic edema. It cannot be decided whether this is caused by hydromechanic pressure due to failure of the right side of the heart or by increased permeability due to anoxemia, to which the liver is sensitive. The latter explanation is supported by the rapid development of edema in drowning or suffocation, although acute heart failure may produce it quickly, too. The marked widening of the perisinusoidal spaces in drowning could also be explained by postmortal osmotic changes due to diffusion of water. Hepatic edema was observed by Eppinger [9] to occur in persons executed by hanging, and Keschner and Klemperer [11] recorded it as occurring after suicidal strangulation.

The rapid development of hepatic edema throws some doubt on its physiologic significance in general and the possible sequelae ascribed to it. It surely speaks against an inflammatory character of this process and against the use of the term "serous hepatitis." The fact that some degree of hepatic edema is found in every person who dies not too suddenly weakens conclusions as to the relation of certain diseases to hepatic edema. Moreover, in contrast to assumptions based on the study of autopsy material, it is absent in many instances of severe hepatic damage studied and cannot, therefore, be considered as a significant factor in these diseases. In some conditions associated with infections and intoxications, the presence of marked toxic edema is not questionable; this is often associated with edema of the gallbladder bed.[18]

Parallel with the widening of the perisinusoidal spaces, the reticulum framework also expands, leading to better visualization of its cross fibers. In contrast with the kidney (in which the reticulum fibers originate from the basement membrane of the tubules and run toward the capillary wall), the fibers in the liver originate from the lining of the sinusoids and run toward the liver cells, the latter having no continuous basement membrane. Most textbook pictures are based on autopsy material, for example, of executed persons, in which, owing to the edema, the fibers are widely expanded. As a further expression of this expansion of the framework in severe edema, the capillary wall may widen as a result of separation of the axial reticulum fibers; this is outspoken when marked edema with much albuminoid material in the tissue spaces compresses the sinusoidal lumen. One could connect the escape of protein into the perisinusoidal space in severe hepatic edema with the thickening of the wall of the capillary produced by the separation of the reticulum fibers, similar to the association of the thickening of the basement membrane of the renal glomerulus with marked albuminuria in nephrosis.[19]

18. Popper.[5] Keschner and Klemperer.[11]

19. Bell, E. T.: Renal Diseases, Philadelphia, Lea & Febiger, 1947.

The widening of the tissue space seems to precede cytoplasmic changes, such as loss of glycogen or of basophilia. Occasionally, the tissue spaces were open when glycogen still caused the liver cells to swell. This speaks against a mere mechanical opening of the tissue spaces by shrinkage of the liver cells due to loss of glycogen. That mechanical factors may influence the width of the tissue spaces is shown by their obliteration in fatty metamorphosis. However, the latter may even interfere with circulation of the blood.[20]

It appears from this study that the condition of the tissue spaces may permit conclusions as to the duration of the agonal period in cases of sudden death. This information may sometimes have medicolegal significance.

SUMMARY

A general comparison of the histologic appearances of the liver in biopsy and autopsy specimens reveals, in addition to cytoplasmic differences—caused primarily by the absence of glycogen from autopsy specimens—that the perisinusoidal tissue spaces are usually closed in biopsy specimens and open in autopsy specimens. Open spaces are associated with an extended reticulum framework such that the cross fibers of the latter are better visualized.

A comparison of a biopsy specimen taken from a liver a few hours before death and an autopsy specimen of the same liver shows that striking dissociation of the liver cell cords may occur in the agonal period. Since this is rarely seen in biopsy specimens, even in cases of severe damage of the liver, regurgitation jaundice cannot be explained by communications between bile capillaries and perisinusoidal spaces which are the result of this dissociation. Whereas in livers of persons dying instantaneously the tissue spaces are obliterated, they may be wide open after an agonal period of longer than 10 minutes—for instance, if sudden death results from suffocation, strangulation or heart failure. This observation speaks against the clinical significance of the hepatic edema of serous hepatitis seen in autopsy specimens, the more so since it is usually absent from biopsy specimens, even in the presence of severe damage of the liver.

The condition of the tissue spaces may be helpful in estimating the duration of the agonal period in cases of sudden death.

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20. Baxter, J. M.: Federation Proc. **7**:145, 1948.

MECHANISMS OF LEUKOPENIA WITH INFLAMMATION

An Additional Leukopenic Factor Found in Alkaline Exudates

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IN PREVIOUS studies I [1] have shown that there is present in inflammatory exudates a leukocytosis-promoting factor (abbreviated as the L.P.F.). This factor is recovered from the pseudoglobulin fraction of exudates.[2] Recently it has been found to be distributed between the α_1 and α_2 globulins of exudates.[3] The present scheme of extraction has been described in recent publications.[4] The active group in the leukocytosis-promoting factor of exudates is presumably a polypeptide group attached to the whole globulin molecule.[5] This group splits off from the rest of the molecule on aging of the material containing the leukocytosis-promoting factor. The leukocytosis-promoting factor helps to explain the mechanism of the leukocytosis frequently associated with inflammation.

The material when freshly extracted from exudates is thermolabile, and it is soluble in an aqueous medium. The object of this communication is to show that when the leukocytosis-promoting factor has been aged for several months it presumably denatures spontaneously and, as a consequence, becomes insoluble in distilled water or saline solution. On centrifugation of the preparation, the supernatant phase is found to contain the split-off leukocytosis-promoting factor, whereas the insoluble part, on the contrary, contains a leukopenic component. The opposing effects elicited by these two fractions result in relative inactivity of the aged leukocytosis-promoting material. Furthermore, it has been often found that during the procedure by which the leukocytosis-promoting material is fractionated from exudates, a fraction that is discarded to obtain a more effective product contains the same leukopenic com-

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From the Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine.

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1. Menkin, V.: Am. J. Path. **16**:13, 1940.
2. Menkin, V.: Arch. Path. **30**:363, 1940.
3. Dillon, M. L.; Cooper, G. R., and Menkin, V.: Proc. Soc. Exper. Biol. & Med. **65**:187, 1947.
4. Menkin, V.: (*a*) Arch. Path. **41**:376, 1946; (*b*) Lancet **1**:660, 1947.
5. Menkin, V.: Blood **3**:939, 1948.

ponent which is found in abundance in aged leukocytosis-promoting material.[4b] Evidently aging the material produces more of this seemingly denatured leukopenic component. Finally, whole exudate when usually alkaline in nature contains this same leukopenic property in the initial stages of its action after it has been administered to to normal dogs. This leukopenic component is thermolabile in contrast to the thermostable leukopenic factor previously described and found also, as a rule, in greater quantities in acid exudates.[6]

TABLE 1.—*Effect of Freshly Prepared Leukocytosis-Promoting Factor on the Number of White Blood Cells*

No. and Date of Preparation of L.P.F.	Dog	Date L.P.F. Was Administered and Amount	White Cell Count Before Injection of L.P.F.	White Cell Counts at Approximate Intervals After L.P.F. Was Injected into Circulation				
				1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.
58-T of 5/ 1/47	11-T	5/ 2/47—42 mg.	11,150	12,450	15,450	28,150	20,500	.....
51-T of 4/22/47	8-D	4/25/47—44 mg.	8,775	14,100	15,450	17,000	11,150	10,850
30-T of 1/29/47	16-T	1/29/47—25 mg.	12,075	19,900	16,500	22,500	32,000	.....
Average			10,667	15,483	15,800	22,550	21,217	.....

TABLE 2.—*Effect of Aged Leukocytosis-Promoting Factor on the Number of White Blood Cells*

No. and Date of Preparation of L.P.F.	Dog	Date L.P.F. Was Administered and Amount	White Cell Count Before Injection of L.P.F.	White Cell Counts at Approximate Intervals After Aged L.P.F. Was Injected into Circulation				
				1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.
58-T of 5/ 1/47	8-D	10/17/47—138 mg.	9,450	1,050	.....	2,850	.....	5,700
51-T of 4/22/47	60-T	11/11/47— 50 mg.	11,650	11,350	9,950	10,700	17,700	12,050
30-T of 1/29/47	8-D	11/14/47— 53 mg.	8,200	6,800	10,050	7,700	8,300	11,000
Average			9,767	6,233	10,000	7,083	13,000	9,583

EXPERIMENTAL PROCEDURE

Dogs are used, and 1.5 cc. of turpentine is injected into the right pleural cavity as described in an earlier communication.[7] Within about twenty-four hours an acute inflammation usually develops. The exudative material withdrawn from such a cavity is, as a rule, alkaline in nature. The leukocytosis-promoting substance is extracted from the exudative material as described previously.[4] The substance is soluble in isotonic sodium chloride solution, and in this solvent it is injected in varying concentrations into the hearts of normal dogs. The results of three such experiments are assembled in table 1. When the leukocytosis-promoting extract is kept at room temperature in a desiccator under phosphoric anhydride for several months, a change in some of its properties occurs, and it becomes relatively inactive. The now aged luekocytosis-promoting substance is found insoluble in an aqueous medium, and it is biologically relatively inert. In fact, it may in certain instances give rise to an initial leukopenia, which is detectable

6. Menkin, V.: Arch. Path. **41**:50, 1946.
7. Menkin, V.: Am. J. Path. **10**:193, 1934.

in the first hour after its administration. Observations relating to this are summarized in table 2, and the course of an individual experiment is graphically illustrated in chart 1. It is quite clear that there is a marked difference between the effect of the freshly prepared leukocytosis-promoting factor and that of the aged preparation.

When aged leukocytosis-promoting material is suspended in an aqueous medium and the suspension is subsequently centrifuged, the supernatant phase is seen to contain the active leukocytosis-promoting factor. In earlier studies this has been shown to be referable to a simple polypeptide which splits off from the rest of the globulin molecule during the probable process of denaturing as the material ages.[5] The observations relating to this appear in table 3. It is seen that with the exception of two experiments there is no initial leukopenic tendency after administration of this supernatant fraction. The average of seven experiments fails to show any initial drop in the total white cell count

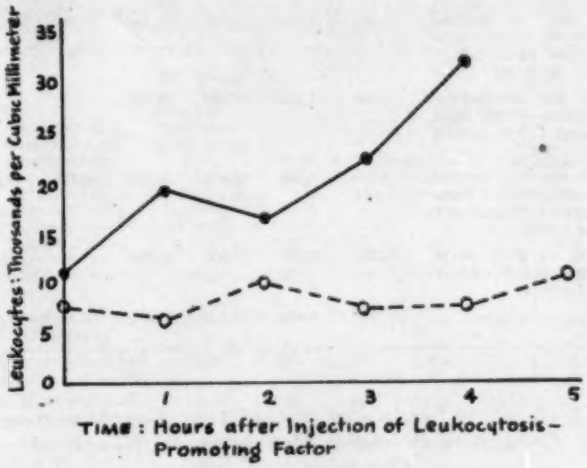

Chart 1.—Effect of aging of the leukocytosis-promoting factor. The solid line represents the number of leukocytes per cubic millimeter of blood following injection of a fresh preparation of the leukocytosis-promoting factor; the broken line, the number following injection of the aged preparation.

(table 3). On the contrary, when the residual or precipitated material from aged leukocytosis-promoting material is studied, it is seen that there is a leukopenic component especially conspicuous during the first hour after administration of this insoluble precipitate (table 4). The dissociation is not perfect, and after an interval of about two or three hours the original level of circulating leukocytes is restored and actually begins to rise as in the case of the supernatant phase (compare tables 3 and 4). Nevertheless, it is this initial leukopenic tendency on the part of the precipitate of the aged leukocytosis-promoting factor that seems to counteract the opposed activity exerted by the supernatant fraction.

The question immediately arises whether this leukopenic component present in the precipitated fraction of aged leukocytosis-promoting material is similar in character to the leukopenic factor described to be present in appreciable amounts in acid exudates.[6] The leukopenic factor of acid exudates is thermostable, and it is closely linked to pyrexin, from which it can in turn be dissociated by

TABLE 3.—*Effect of Supernatant Fraction (Probably Polypeptide in Nature) from Aged Leukocytosis-Promoting Factor on the Number of Circulating Leukocytes*

Dog and Date	Amount of Dried Supernatant Fraction Injected, Obtained from Aged L.P.F.	White Cell Count Before Injection of Supernatant Fraction	White Cell Counts at Approximate Intervals After Supernatant Fraction Was Injected into Circulation					
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
63-T 10/23/47	17 mg. from aged L.P.F. of 58-T of 5/1/47	7,250	12,400	15,150	17,650	13,300	.....	.....
11-T 10/24/47	50 mg. from aged L.P.F. of 58-T of 5/1/47	12,600	11,500	21,950	17,800	19,950	.....	.....
32-T 10/30/47	38 mg. from aged L.P.F. of 58-T of 5/1/47	5,450	8,700	11,250	16,400	12,650	.....	.....
54-T 11/ 5/47	27 mg. in normal canine blood serum from aged L.P.F. of 58-T of 5/1/47	7,100	9,200	8,900	15,450	15,300	15,950	.....
31-T 11/ 6/47	36.5 mg. in normal canine serum from aged L.P.F. of 58-T of 5/1/47	7,550	7,300	11,600	13,350	.....	.....	.....
63-T 11/10/47	31.9 mg. in normal canine serum from aged L.P.F. of 58-T of 5/1/47	12,750	8,600	12,450	18,150	22,150	18,350	.....
60-T 11/12/47	5 cc. of fluid from aged L.P.F. of 51-T of 4/22/47	9,750	12,250	10,500	13,700	15,000	19,200	21,750
Average.....		8,921	9,993	13,114	16,071	16,392	17,833	.....

TABLE 4.—*Effect of Precipitated or Leukopenic Component from Aged Leukocytosis-Promoting Material on the Number of Circulating Leukocytes*

Dog	Amount of Suspension of Precipitate* from Aged L.P.F.	White Cell Count Before Injection of Precipitated Component of Aged L.P.F.	White Cell Counts at Approximate Intervals After Precipitated Component Was Injected into Circulation						
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.	7 Hr.
63-T	— cc.† from 58-T of 5/1/47	13,150	8,650	10,450	10,950	.....	.....	.....	.....
54-T	3.5 cc. from 58-T of 5/1/47	7,300	4,900	4,850	8,200	10,150	9,250	15,000	11,750
8-D	7.0 cc. from 58-T of 5/1/47	5,250	2,550	4,600	8,150	6,400	.....	.....	.....
60-T	10-11 cc. from 51-T of 4/22/47	10,450	7,200	7,850	.....	10,600	10,900	.....	.....
11-T	15 cc. from 30-T of 1/29/47	13,400	8,050	12,800	12,550	9,850	.....	12,000	.....
Average.....		9,910	5,270	8,110	9,963	9,250	10,075	13,800	.....

* The precipitated component was suspended in saline solution.
† The actual amount was not recorded.

incomplete hydrolysis with tenth-normal hydrochloric acid.[6] Under such circumstances the potency of the leukopenic factor remains essentially unaltered.[6] In the present study the leukopenic component of aged leukocytosis-promoting material is inactivated by incomplete hydrolysis with tenth-normal hydrochloric acid. The observations relating to this are shown in table 5. It is evident from table 5 that with the exception of two questionable experiments the leukopenic component of aged leukocytosis-promoting material is wholly inactivated by incomplete acid hydrolysis. The average figures indicate that there is no initial leukopenia following administration of the product of incomplete hydrolysis of aged leukocytosis-promoting material. This finding points out the difference in the two separate leukopenic components obtained from exudative material. Furthermore, the results obtained are not referable to the partial hydrolysis in itself, for when the process of hydrolysis is performed with tenth-normal hydrochloric

TABLE 5.—*Inactivation of the Leukopenic Effect from the Precipitated Component of Aged Leukocytosis-Promoting Material by Incomplete Hydrolysis of That Component*

Dog and Date	Amount of Suspension of Incompletely Hydrolyzed Precipitate from a Given Amount of Aged L.P.F.	White Cell Count Before Injection of Incompletely Hydrolyzed Precipitated Component of Aged L.P.F.	White Cell Counts at Approximate Intervals After Incompletely Hydrolyzed Precipitated Component Was Injected into Circulation					
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
46-T 12/ 4/47	10 cc. from 86 mg. of 58-T of 5/1/47	8,675	15,700	17,600	14,800	12,900	.....	15,800
60-T 12/ 5/47	7 cc. from 60 mg. of 58-T of 5/1/47	16,525	15,900	20,350	.....	14,850	.....	18,966
63-T 12/ 6/47	12 cc. from about 45 mg. of 51-T of 4/22/47	11,800	11,500	15,200	19,600	.....	.....	.....
54-T 12/ 8/47	10 cc. from about 44 mg. of 51-T of 4/22/47	10,450	14,900	14,400	15,200	14,200	15,550	14,200
8-D 12/10/47	14 cc. from 44 mg. of 58-T of 5/1/47	8,783	9,200	11,250	9,863	10,000	9,975	.....
54-T 12/11/47	19 cc. from 140 mg. of 51-T of 4/22/47	13,583	18,850	13,300	16,000	13,900	.....	.....
Average.....		11,636	14,342	15,350	15,093	13,170	12,763	16,317

acid only, there is no essential change in the number of circulating leukocytes on injection of such material.[6] The exclusive leukocytosis following partial hydrolysis of the precipitated component of aged leukocytosis-promoting material is doubtless referable to the heat-stable polypeptide in the aged leukocytosis-promoting factor. This factor is usually found in the supernatant phase, but it can also be present to some extent in the precipitated part of the aged material (table 4).

With the foregoing finding that a leukopenic component is present in the insoluble part of aged leukocytosis-promoting material, the question arises as to the presence or the absence of such a leukopenic factor in freshly withdrawn whole exudate. When such an exudate is injected into the circulation of a normal dog, there is initial leukopenia, soon followed by leukocytosis. The latter is referable to the leukocytosis-promoting factor present in the exudate.[1] At the time that the leukocytosis-promoting factor was first demonstrated to exist in exudates, it was pointed out that at first there is transitory leukopenia.[1]

This state of affairs recalled the leukopenia described by Ewing [8] and by Webb [9] which occurs during anaphylactic shock. Several years later a similar leukopenic phase was said to occur with improperly purified leukocytosis-promoting factor.[10]

TABLE 6.—*The Possible Presence in Exudates of a Thermolabile Factor, Besides the Thermostable Leukopenic Factor, Capable of Lowering the Number of Circulating Leukocytes.*

Dog	p_H of Exudate	Amount of Exudate Injected into the Circulation, Cc.	White Cell Count Before Injection of Exudate	White Cell Count at Approximately One Hour After the Injection of the Exudate
26-D	7.4	5	10,500	6,550
52-D	7.5	9-10 Administered in dried form in saline solution, this being the equivalent of 514 mg. of lyophilized exudate	8,175	3,700
60-T	7.2	4	15,300	12,450
52-D	7.5	5	15,350	9,450 2 hours after the injection of the exudate
54-T	7.2	4	9,900	6,150
87-T	7.5	4.5	9,225	6,600
87-T	7.5	5	9,350	7,550
87-T	7.5	4.5	6,075	6,950 2 hours after the injection of the exudate
87-T*	7.5	2	7,550	10,550
Average	...	..	10,158	7,773 Per cent reduction = 23.5
22-D†	7.5	5	19,375	16,000 2 hours after injection of the boiled exudate
60-T†	7.2	4.5	19,900	18,550
63-T†	7.2	4	12,875	12,400
87-T†	7.5	5	10,825	7,150
85-T†	7.5	5	12,900	10,000
91-T†	7.5	4.5 to 5	15,400	13,200
91-T†	7.5	5	15,675	13,100
Average	...	..	15,279	12,914 Per cent reduction = 15.5
63-T‡	6.0	3	11,625	8,150
87-T	6.0-6.2	5	9,250	7,050
94-T‡	6.0	5	11,350	9,450
Average	...	..	10,742	8,217 Per cent reduction = 23.5
54-T§	6.0	3	13,675	3,500
91-T†	6.0-6.2	5	11,050	10,050
85-T§	6.0	4.5	11,500	8,150
Average	...	..	12,075	7,333 Per cent reduction = 39

* Any leukopenic effect was possibly masked by the effect of the leukocytosis-promoting factor in the sample of exudate.

† This sample of exudative material was brought to the boiling point before being injected into the circulation of the animal.

‡ This sample of exudate was removed at postmortem examination from the chest of an animal that had been given a second injection of the irritant and as a consequence was very ill.

§ The foregoing explanation holds true of the material injected, but in addition it was boiled.

8. Ewing, J.: New York M. J. **61**:257, 1895.
9. Webb, R. A.: J. Path. & Bact. **27**:79, 1924.
10. Menkin, V., and Kadish, M. A.: Am. J. M. Sc. **205**:363, 1943.

At that time this was thought to be due to some toxic impurities.[10] In the present study exudative material, some at alkaline and some at acid p_H, was injected into dogs, and thereafter the number of leukocytes per cubic millimeter of blood was studied. It was generally found that when an alkaline exudate (p_H ranging from 7.2 to 7.5) was introduced into the blood of a normal dog, an hour later a drop in the number of circulating leukocytes tended to occur. This reduction averaged 23.5 per cent (table 6). When, however, such exudate was heated to the boiling point, the reduction in white cells was distinctly less, averaging 15.5 per cent. It seems as if heating had destroyed a part of the leukopenic component present in the alkaline exudate. When the exudate was in an acid phase (p_H 6.0 to 6.2), its introduction was likewise followed by an initial drop in

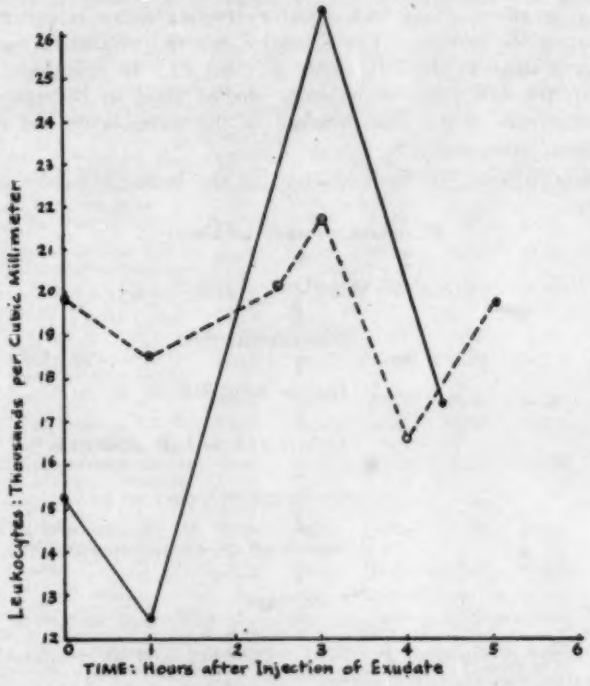

Chart 2.—Effect of boiling of alkaline exudate on the leukopenic components. The solid line represents the number of leukocytes per cubic millimeter of blood following injection of unheated exudate; the broken line, the number following injection of boiled exudate.

the leukocytic level, averaging also 23.5 per cent (table 6). Bringing such acid material to a boil, however, rendered the exudate even more potent as a leukopenic agent. The average reduction now amounted to 39 per cent (table 6). What is the interpretation of these results? It seems as if an alkaline exudate may contain two leukopenic factors. One is thermolabile and the other, probably in smaller quantities, is thermostable. The latter does not appear to be different from the leukopenic factor described in an earlier study.[6] When the alkaline exudate is boiled, the thermolabile factor is inactivated, leaving only the thermostable leukopenic factor. This would account for the reduction in the drop in the white blood cell count from 23.5 to 15.5 per cent, i. e., the reduction

obtained by simply bringing the alkaline exudate to a boil (table 6). On the other hand, an acid exudate contains the heat-stable leukopenic factor usually found in abundance in such exudates, whereas the thermolabile leukopenic factor is present, if at all, in insignificant amounts in acid material. Boiling the acid exudate inactivates the leukocytosis-promoting factor present, since the latter tends to be thermolabile.[1] The consequence is the unobstructed action of the thermostable leukopenic factor, so that the drop of the white blood cell level is even more marked, with a reduction of 39 per cent (table 6). The initial drop in the circulating white cells is shown in chart 2. Subsequently a rise occurs, due to the leukocytosis-promoting factor present in the exudative material (chart 2). Boiling such material and injecting it are followed by an initial drop which is definitely less pronounced than that observed with the untreated exudate (chart 2). The effect of any leukocytosis-promoting factor is essentially eliminated by heating the exudate. The exudative material utilized in this particular experiment was alkaline (p_H 7.2) (table 6; chart 2). In conclusion, the effects obtained with the two types of exudates studied seem to be referable to the respective differences in the concentrations of the thermolabile and the thermostable leukopenic component.[10a]

The present scheme for the extraction of the leukocytosis-promoting factor is as follows:

Exudate (usually alkaline)
↓
$(NH_4)_2SO_4$ to a third saturation
↓
Supernatant fluid
↓
Dialyze until free of SO_4=
↓
$(NH_4)_2SO_4$ to half saturation
↓
Overnight or longer on ice
↓
Siphon off or decant supernatant fluid
↓
Centrifuge

Discard precipitated part (as a rule, this fraction contains relatively little leukocytosis-promoting factor, but it may have traces of a leukopenic factor)	Unsettled part of precipitate ↓ Dialyze until free of SO_4= ↓ Leukocytosis-promoting factor ↓ Dried by freezing (kept for a week or two *in vacuo* under phosphoric anhydride)

10a. Differential absorption by boiling exudates of different initial p_H is an improbable interpretation of the aforesaid results, for in earlier studies it was shown that there is a leukopenic factor which is a heat-stable polypeptide obtained usually at acid p_H and associated with pyrexin,[6] whereas the present factor is usually obtained from alkaline exudates as a thermolabile factor associated with the globulins of these exudates.

It is essential to perform proper lyophilization for about 24 hours. Longer periods may end to denature the material, i. e., as far as biologic potency is concerned. It is best to freeze the material as a fairly thin film in a glass container. Finally, it has been observed that insufficient lyophilization, resulting in thawing, followed then by a second attempt to dry the material, usually yields a relatively inactive product with a leukopenic phase in it. It seems from these remarks that the leukocytosis-promoting factor of exudates is easily denatured and thus may lose its potency if proper precautions are not followed.

As indicated in the scheme of extraction, the final precipitated fraction sometimes contains a leukopenic factor similar to the one found in abundance in aged leukocytosis-promoting material. The observations relating to this appear in table 7. It is quite clear that in 8 of 9 experiments the initial leukopenic

TABLE 7.—*Leukopenic Effect Induced by the Precipitated Fraction Obtained in the Final Extraction of the Leukocytosis-Promoting Material*

Dog	Amount of Precipitated Material Dissolved in an Aqueous Medium	White Cell Count Before Injection of Material	White Cell Counts at Approximate Intervals After Precipitate Fraction Was Injected into Circulation					
			1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
8-D	27 cc. (equivalent to 20 cc. of original exudate)	14,913	8,625	12,350	15,150	17,850	.....	.....
31-D	22 cc. (80 mg. of material)	14,725	6,950	6,850	10,100	12,100	13,750	17,650
22-D	11 cc.	14,650	21,150	5,050	7,350	6,150	5,750	.....
8-D	15 cc. (equivalent to 10 cc. of injected exudate)	13,175	11,100	11,900	18,500	11,100	16,000	15,900
31-D*	11 cc.	18,325	12,000	22,250	23,900	26,150	27,200	28,450
45-D*	13.5 cc. (51.5 mg. L.P.F. and precipitate)	17,000	4,550	11,950	14,600	15,900	40,000	.....
26-D*	22 cc. (equivalent to 20 cc. of original exudate)	12,375	4,450	10,350	13,100	15,150	14,150	.....
46-D*	10 cc.	17,050	5,950	20,100	33,900	.....	.....	.....
22-D†	20 cc. (equivalent to ±18 cc. of original exudate)	20,950	10,550	16,050	18,950	23,900	18,700	20,550
Average.....		15,907	9,481	12,983	17,283	16,038	19,364	20,613

* This dog received the precipitated fraction along with some leukocytosis-promoting factor.
† This dog received, not the precipitated fraction, but rather an initial fraction in the preparation of the leukocytosis-promoting factor. Both the precipitated fraction and the leukocytosis-promoting factor are presumed to have been still present in it.

tendency on the part of this precipitated fraction was present. It is for this reason that in the extraction of an active leukocytosis-promoting factor the precipitate is being discarded (see extractive scheme). This leukopenic fraction extracted from exudates is thermolabile; boiling the material inactivates it (table 8). It is thus seen that this fraction obtained from fresh exudates does not seem to differ in any way from the fraction obtained from the precipitate of the aged leukocytosis-promoting extract (compare table 4 with table 7). It is therefore my belief, in view of these observations, that in alkaline exudates there is a thermolabile leukopenic factor closely associated with the globulins of the leukocytosis-promoting factor. It seems to occur as a consequence of a denaturation of the proteins. At first it occurs with injury to cells, so that it can be recovered in an alkaline exudate. Eventually more of it forms as the leukocytosis-promoting extract is allowed to age for several months. The material, kept in a desiccator under phosphoric anhydride, apparently denatures

spontaneously, loses its original property of being soluble in an aqueous medium, and evidently forms this leukopenic factor in greater abundance. The latter can be recovered as the insoluble part of aged leukocytosis-promoting material. For this thermolabile leukopenic factor, which is found also in alkaline exudates, though to a less extent perhaps, the term "leukopenin" is suggested to distinguish

TABLE 8.—*The Thermolability of the Precipitated Fraction Obtained in the Extraction of the Leukocytosis-Promoting Factor*

Dog	Initial White Cell Count	White Cell Count One Hour After Intravenous Injection of Precipitated Fraction (Heated to Boiling)
84-T*	14,075	14,400
91-T*	13,425	12,350
87-T	6,450	10,050
94-T	10,025	13,100
85-T	11,175	11,850
Average	11,050	12,750

* The precipitated fraction was refluxed for about 10 minutes with tenth-normal hydrochloric acid, neutralized with normal sodium hydroxide, and dialyzed to rule out the presence of the leukopenic factor.

TABLE 9.—*Initial Effect of Leukocytosis-Promoting Factor* Obtained from Human Exudate on the Number of Circulating Leukocytes*

Dog	Number of Leukocytes per Cubic Millimeter of Blood Prior to Injection of Material	Initial Drop in White Cell Count Following Injection of Material
7-31	9,900	4,000*
7-31	11,650	2,650 (Essentially no euglobulin admixed to L.P.F.)
7-31†	18,200‡	4,500‡
7-33§	15,900	12,000‡
7-29	18,400	4,700 (Essentially no euglobulin fraction in this material)
7-34§	7,150	5,000 (Essentially no euglobulin fraction in this material)
Average	13,533	5,475

* This leukocytosis-promoting fraction of human exudate contained the entire globulin content of the exudate. (The method utilized has been described by Menkin and Kadish.[10])

† Fifty cubic centimeters of whole exudate was used instead of the leukocytosis-promoting extract.

‡ This figure is a first approximation inasmuch as it was interpolated from a graphic representation of these experiments.

§ The leukocytosis-promoting material from a human source was injected subcutaneously; in other experiments it was injected intravascularly.

it from the leukopenic factor which is closely associated with pyrexin and which is thermostable.[6]

In a recent discussion Hadfield and Garrod[11] expressed the view that in the conclusions drawn by me there may be an element of generalization; perhaps a

11. Hadfield, G., and Garrod, L. P.: Recent Advances in Pathology, ed. 5, Philadelphia, The Blakiston Company, 1947.

similar attitude has been pointed out by Wilson.[12] These views do not seem wholly warranted, for leukotaxine has been found in the exudates of several different species of animals.[13] The leukocytosis-promoting factor has been identified in dogs,[1] rabbits[14] and man.[15] Finally, necrosin has been studied both in dogs and in man.[16] Nevertheless, in view of these criticisms a study was made in an endeavor to find out whether leukopenin was also present in another species, namely, man. An initial leukopenic tendency had been noted previously as occurring both in human exudate and in a leukocytosis-promoting extract of human exudate.[10] The observations relating to this are assembled in table 9, and the course of an experiment with human material is graphically illustrated

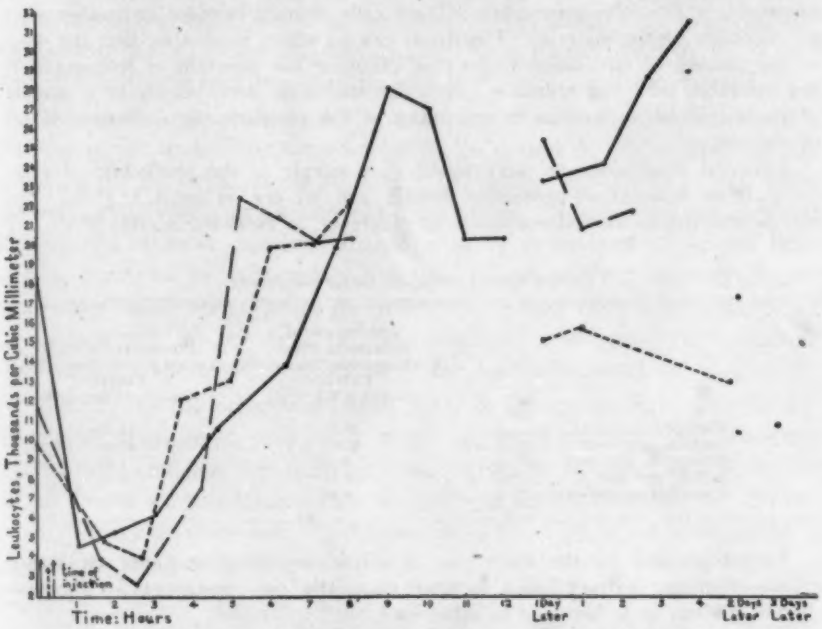

Chart 3.—Effect of the human leukocytosis-promoting factor. One curve (———) represents the number of leukocytes per cubic millimeter of blood of a dog (7-31) after the dog was given an injection of whole exudate (50 cc.) obtained from a patient; a second curve (— — —), the number after the dog was given a desiccated leukocytosis-promoting extract (obtained from approximately 31 cc. of the whole exudate); a third curve (- - - - - -), the number after the dog was given 100 mg. of the dessicated extract.[10]

in chart 3. It is quite clear from the data that a similar leukopenic trend is found soon after administration of human exudate or of an extract of it con-

12. Wilson, G. S.: Principles of Bacteriology and Immunity, ed. 3, Baltimore, Williams & Wilkins Company, 1946.

13. Menkin, V.: Dynamics of Inflammation, New York, The Macmillan Company, 1940. Cullumbine, H., and Rydon, H. N.: Brit. J. Exper. Path. **27**:33, 1946

14. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **64**:448, 1947.

15. Menkin, V.; Kadish, M. A., and Sommers, S. C.: Arch. Path. **33**:188, 1942.

16. Menkin, V.: Arch. Path. **36**:269, 1943.

taining the leukocytosis-promoting factor. These results, in addition to the ones obtained with other substances from exudates, would somewhat weaken the contention of excessive generalization based on only meager factual information.

It is also of interest to know the type of cells affected by the leukopenin recovered from alkaline exudates. In the initial drop following the intravascular injection of leukopenin the mononuclear cells are primarily involved. This includes a combination of both the lymphocytes and the monocytes. In a few instances one of these two types of cells is more involved than the other; but in general both lymphocytes and monocytes are affected, i. e., in over 80 per cent of the cases studied. In about 50 per cent of the observations the polymorphonuclear neutrophils are also involved. The fall does not seem to affect the immature, or one lobe, neutrophils. These cells steadily increase in number after the injection of the material. Finally, it can be stated here, also, that the drop in the number of circulating leukocytes following the injection of leukopenin is not referable to a redistribution effect, for the same drop occurs in a sample of cardiac blood as well as in one taken at the periphery by nicking a vessel of the ear.

Chemical determinations were made on a sample of the precipitate of relatively fresh leukocytosis-promoting extract and on one of aged L. P. F. The two determinations showed essentially no difference, as indicated in table 10.

TABLE 10.—*Chemical Determinations*

	Precipitate of Relatively Fresh Leukocytosis-Promoting Extract (About 3 Wk. Old)	Precipitate of Aged Leukocytosis-Promoting Extract (About 15 Mo. Old)
Nitrogen, per cent	10.55	11.46
Carbon, per cent	43.88	46.44
Hydrogen, per cent	6.85	7.40
Sulfur, per cent	0.51	1.37
Phosphorus, per cent	0.33	....

Except perhaps for the slight rise in sulfur concentration, aging the leukocytosis-promoting extract failed to alter materially the constituents of the precipitate studied, as is indicated in table 10.

COMMENT

These studies indicate that exudative material contains at least two factors concerned in the mechanisms of leukopenia with inflammation. There is in alkaline exudates, associated with the globulins of the leukocytosis-promoting factor, a thermolabile leukopenic factor. This leukopenic component appears to be a product of protein denaturation concomitant with cell injury, for it seems to be found in appreciable quantity by merely allowing an active preparation of leukocytosis-promoting factor, at first devoid of it, to age for several months.[1] A spontaneous denaturation occurs. The leukocytosis-producing extract becomes insoluble in an aqueous medium, and in the precipitated part the leukopenic component can be recovered. With fresh exudates the component is found present in an unused fraction obtained in the preparation of an active leukocytosis-producing extract. The chemical

constituents of the insoluble fraction of aged leukocytosis-promoting extract and the unused fraction obtained at the time of recovery of fresh leukocytosis-promoting extract are essentially similar. The term "leukopenin" is suggested for this leukopenic component identified particularly in alkaline exudates.

In alkaline exudates there is also present the thermostable leukopenic factor previously described.[6] In such exudates it is recovered in relatively small quantities. It is found, however, in larger abundance in acid exudates. This is not fully surprising, for the latter leukopenic component has been found to be closely associated with pyrexin, the pyrogenic factor. Pyrexin, in turn, has been found to occur more frequently and in larger amounts in acid than in alkaline exudates.[17]

Whether leukopenin acts by trapping the leukocytes in various tissues is yet to be determined.[18] The reduction of the number of circulating white cells seems to affect primarily the mononuclear cells, namely, the lymphocytes and the monocytes. Sometimes one type of these cells is more involved than the other type, but in general both types seem to be affected. The polymorphonuclear leukocytes are depressed in number during the leukopenic phase caused by leukopenin in only about 50 per cent of the cases. Further study is necessary to determine the exact mechanism of the reduction involved.

Finally, to the other substances listed in an earlier communication,[19] liberated by injured cells as a result of their impaired biochemistry, leukopenin can also be added. This factor, in addition to the leukopenic factor of exudates, helps in one's understanding of the mechanisms of leukopenia with inflammation. None of these substances is present in normal blood serum.[20]

17. Menkin, V.: Federation Proc. **4**:149, 1945.

18. Menkin, V.: Arch. Path. **42**:154, 1946.

19. Menkin, V.: Science **105**:538, 1947.

20. The view has been expressed that the present and the previous substances isolated from exudates are crude mixtures and therefore that their meaning may be somewhat doubtful. This type of criticism fails to take into consideration certain facts. The whole exudate, as such, is shown to possess certain biologic properties. These properties are not present in normal blood serum. Further analysis of the whole exudate yields biochemical units which have precisely the same properties as the whole exudate. To be certain, it would be desirable to know the exact chemical formulation of these units. It is hoped that future studies will yield such data; but present investigators do not seem quite ready for such studies; or at least these have not yet been undertaken by specialized chemists. This has always been the history of almost any biologic substance derived either from animal or from plant sources. At first the substance is obtained in a relatively crude form. Subsequently it is purified to its final form. The studies on the leukocytosis-promoting factor have progressed somewhat in this direction, for the active grouping has already been ascertained to be a relatively simple polypeptide.

SUMMARY AND CONCLUSIONS

There is present in exudates, particularly those that are alkaline in nature, a leukopenic component closely associated with the globulins of the leukocytosis-promoting factor. This leukopenic component is thermolabile. This distinguishes it from the thermostable leukopenic factor previously described as recovered from acid exudates.[6] The leukopenic component of exudates associated with the globulins of the leukocytosis-promoting factor seems to be a product of a protein denaturation following the initial injury of cells with the onset of inflammation. Aging the leukocytosis-promoting factor induces the further production of this leukopenic component, presumably by spontaneous denaturation. The formation of this factor tends to reduce the potency of, or even to inactivate, the usual leukocytosis-promoting factor, the factor which accelerates the discharging of polymorphonuclear leukocytes into the blood stream. To render the leukocytosis-promoting factor extracted from freshly withdrawn exudates more effective, the leukopenic component, particularly that found in alkaline exudates, is eliminated in the scheme of extraction of the leukocytosis-promoting factor.[4b]

Earlier studies have demonstrated that a thermostable leukopenic factor is present in exudates.[6] The knowledge that the thermolabile leukopenic component of exudates is in combination with the thermostable one helps in one's understanding of the mechanisms of leukopenia with inflammation.

The initial leukopenia induced by the thermolabile leukopenic component of exudates affects primarily the mononuclear type of white cells and to some extent the polymorphonuclear leukocytes.

The term "leukopenin" is suggested for this additional leukopenic component concerned in the mechanism of leukopenia with inflammation.

SIGNIFICANCE OF THE BETA GRANULES IN THE ISLETS OF LANGERHANS OF THE PANCREAS

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THE ISLETS of Langerhans are composed of two distinct types of cells, alpha and beta cells, which may be distinguished by appropriate staining. My associates and I have used Gömöri's stain exclusively, since it is easy to apply and gives consistent results. With this stain the alpha cells are colored red, and their cytoplasm shows no distinct granules; but the beta cells contain numerous small blue particles, which are called beta granules (fig. 1).

It is now well established that insulin is formed by the beta cells. Injections of alloxan cause complete necrosis of all the beta cells without injury to the alpha cells, and the animal becomes permanently diabetic. The adenoma of the pancreas which causes hyperinsulinism is composed of cells which contain some beta granules.

Beta granules are greatly reduced in number or are entirely absent in the human diabetic pancreas in about two thirds of the cases. Degranulated beta cells of the human pancreas show no other evidence of injury, and the significance of degranulation is not understood.

The purpose of this investigation was to determine whether the beta cells may be degranulated by experimental procedures which decrease the demand for insulin, such as fasting, feeding a diet consisting exclusively of fat or daily administration of insulin.

Best, Haist and Ridout,[1] by means of extraction and quantitative assays, found definite reductions in the insulin content of the pancreas of rats after subjecting these animals to fasting, after keeping them for a period on a fat diet and after administration of insulin. They pooled the pancreases of 10 normal rats and found the total insulin content to be 26.5 units. After a fasting period of seven days the insulin content of the pancreas of 10 animals was 14.1 units. Rats fed a normal diet and given a daily injection of protamine zinc insulin showed an insulin content of 10 units per 10 rats after a period of seven days.

From the Department of Pathology, University of Minnesota.

This investigation was supported by a grant made to Dr. E. T. Bell by the Office of Naval Research.

1. Best, C. H.; Haist, R. E., and Ridout, J. H.: Diet and the Insulin Content of the Pancreas, J. Physiol. **97**:107, 1939.

Rats fed a diet 90 per cent fat for seven days showed an insulin content of 12.3 units per 10 pancreases. But when insulin was administered daily to rats on the fat diet, the insulin content was reduced to 3.8 and 4.1 units per 10 pancreases after a period of seven days.

These investigators expressed the belief that under these experimental conditions the reductions of insulin were due to decreases of demand occasioned by the lack of carbohydrate and by the exogenous supply of insulin, respectively. The islets were put at rest and apparently formed less insulin. In one experiment they found a decrease of beta granules in pancreases with a low content of insulin, suggesting

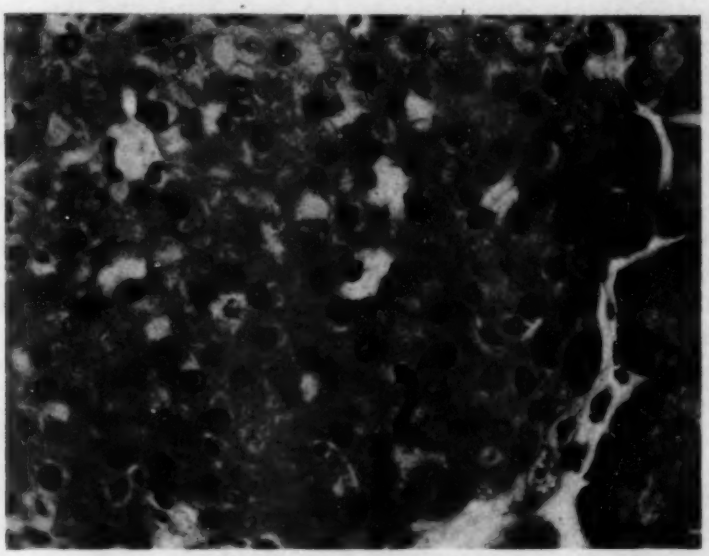

Fig. 1.—Islet of Langerhans from the pancreas of a normal rat, stained by Gömöri's method. The beta cells are filled with small granules, which are colored a deep blue in the original preparation.

a direct correlation of beta granules and insulin content. However, to demonstrate beta granules, these investigators used Bowie's stain, which is not as satisfactory as Gömöri's stain.

MATERIAL AND METHODS

White rats were used for all of the experiments of the present study, and the animals were divided into four groups. Group 1 consisted of fasted rats; group 2, of rats maintained on lard; group 3, of rats fed olive oil exclusively, and group 4, of rats fed a regular diet and given a daily injection of protamine zinc insulin. The pancreas was fixed in Bouin's fluid, and paraffin sections were stained by Gömöri's method.

Group 1. Fasting Rats (table 1).—The 13 rats of this group were kept in separate cages and were allowed a free supply of water but were given no food. A number of rats were lost because they died during the night and the subsequent postmortem changes prevented a histologic study of the pancreas. Only those animals are included which were killed at the end of the experimental period.

TABLE 1.—*Fasting Rats*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47- 62	215	...	3	3
47- 63	275	...	5	3
47- 64	270	...	5	2
47- 65	270	...	5	3
48-209	160	95	8	0
48-208	215	120	8	1
48-206	155	110	8	2
48-205	120	85	8	1
48-201	190	119	8	3
48-202	175	103	8	1
48-203	160	100	8	1
48-207	160	105	8	2
48-210	160	95	8	3

It will be noted in table 1 that there was a marked loss of body weight. The numerals 0 to 3 indicate the amount of beta granulation. Grade 0 means that no granules were demonstrable, and grade 1 indicates a striking reduction of granules. Grade 2 means a moderate decrease of granules within the limits of normal

TABLE 2.—*Rats Fed on Lard*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47-154	230	160	13	1—
47-155	185	110	13	1—
47-156	200	130	13	1—
47-157	210	145	13	0
47-158	230	170	13	1—
47-159	225	160	13	1—
47-160	150	100	13	0
47-161	125	75	13	1—
47-162	125	75	13	0
47-163	125	80	13	1—
47-164	140	85	13	1—
47-165	125	75	13	1—
48-211	155	105	9	1—
48-212	150	100	9	0
48-213	120	80	9	0
48-214	170	125	9	1
48-215	210	115	9	0
48-216	160	110	9	1
48-217	185	135	9	1—
48-218	175	135	9	1
48-219	140	95	9	1—
48-220	170	115	9	1

variation, and grade 3 indicates normal granulation. A fasting period of five days or less did not affect the beta granules, but a fast of eight days caused complete degranulation in 1 rat and marked reduction of granules in 4 others, but in 4 animals the granules were unaffected by fasting for eight days.

Group 2. Rats Fed on Lard (table 2).—The 22 rats of this group were maintained on a diet consisting exclusively of lard. They had free access to water. Ten animals were killed at the end of a 9 day period, and 12 after 13 days. There was a striking loss of body weight, especially in the group maintained for 13 days. The degree of degranulation was about as marked in the 9 day as in the 13 day group. In 6 animals the beta cells were completely degranulated (grade 0) (fig. 2); in 12 animals there were only occasional beta granules (grade 1—), and in 4 there was definite reduction of granules but it was not so severe as in the others (grade 1).

Best and Haist [2] found that rats maintained for seven days on a diet 90 per cent fat showed the insulin of the pancreas reduced to about 50 per cent of normal. Possibly the insulin content would have been reduced more if the experiment had been continued longer.

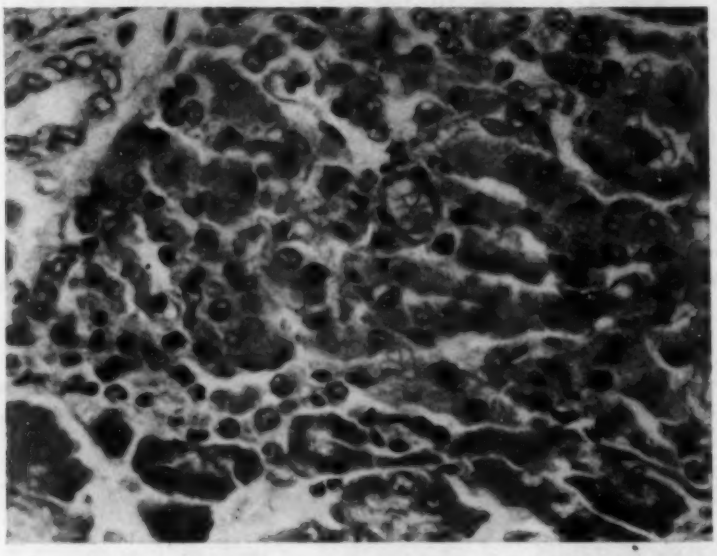

Fig. 2.—Islet of Langerhans from the pancreas of a rat maintained exclusively on lard for 13 days. The beta cells are completely degranulated. Photomicrograph.

Group 3. Rats Fed on Olive Oil (table 3).—The 17 rats of this group were fed olive oil exclusively but were allowed free access to water. Nine of the 17 rats were killed after 20 days of the diet, and the others after 23 days. There was a severe loss of weight in every animal. In 6 rats there was complete degranulation of the beta cells (grade 0), and in 7 others only occasional beta granules were found (grade 1—). In the remaining 4 rats the degranulation was marked but was not so severe as in the others (grade 1).

Group 4. Rats Treated with Insulin (table 4).—The animals of this group were maintained on the regular diet without any restrictions. Each rat was given a daily injection of 4 to 6 units of protamine zinc insulin. The animals ate

2. Best, C. H., and Haist, R. E.: The Effect of Insulin Administration on the Insulin Content of the Pancreas, J. Physiol. **100**:142, 1941.

well and showed no clinical disturbances. They either maintained their body weight or grew heavier. Three rats killed after one week showed severe degranulation of the beta cells. In the cases of 2 rats a biopsy of the pancreas made after two months of insulin injections showed complete degranulation of the beta cells. There was no evidence of atrophy or of degeneration of the beta cells after two months, and no indication that this treatment would ultimately produce diabetes.

TABLE 3.—*Rats Fed on Olive Oil*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47-134	175	130	23	1—
47-135	130	80	23	0
47-136	175	110	23	0
47-137	125	65	23	1—
47-138	150	105	23	0
47-139	155	100	23	1—
47-140	150	110	23	1
47-141	140	85	23	1—
48-221	150	85	20	1—
48-222	130	65	20	0
48-223	140	80	20	0
48-224	130	55	20	0
48-226	165	90	20	1
48-227	190	105	20	1
48-228	185	95	20	1—
48-229	135	60	20	1—
48-230	200	105	20	1

TABLE 4.—*Rats Treated with Insulin*

Serial No.	Initial Weight, Gm.	Final Weight, Gm.	Duration, Days	Beta Granules
47-186	120	...	45	0
47-192	170	275	Biopsy, 2 mo.	0
48-232	145	135	7	1—
48-236	145	140	7	1
48-234	140	135	7	0
47-189	220	270	Biopsy, 2 mo.	0

SUMMARY

The three experimental procedures outlined, viz., fasting, maintenance on a diet restricted to fat and daily administration of insulin, all produce degranulation of the beta cells. Fasting is less effective in producing degranulation than the other procedures, probably because the animals of this group did not live so long. When there is no carbohydrate in the diet, insulin is not required, and one may believe that insulin is not produced when it is not needed. The work of Best and Haist showed that the insulin content of the pancreas is decreased by the procedures outlined. Since there is a direct correlation between the insulin content of the pancreas and the number of beta granules, the conclusion seems justified that the beta granules represent a precursor of insulin.

NUCLEIC ACIDS AND CYTOLOGIC CHANGES IN REGENERATING RAT LIVER

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THERE is considerable recent evidence that the nucleic acids of cells play an important role in the formation of cellular proteins and that the nucleolus with its associated chromatin is one of the vital cell structures participating in this function.[1] To obtain further information concerning these relationships, experiments were undertaken employing liver tissue, which has large nucleoli in cells capable of remarkably rapid regenerative growth. The changes in nucleic acids as determined by special cytochemical and macrochemical methods were correlated with morphologic changes including the mean sizes of the nucleolus, the nucleus and the cytoplasm of the hepatic cell.[2]

Detailed ultraviolet cytochemical observations[3] on a large variety of types of living cells indicate that the nucleolus-associated chromatin (heterochromatin) produces protein substances which constitute, in part at least, the nucleolus. In conjunction with these nucleolar materials the nuclear membrane produces ribose nucleic acids leading to increased cytoplasmic protein. In favorable material a nucleic acid gradient from the nucleolus toward the nuclear membrane as well as from the membrane into the surrounding cytoplasm has been shown. This evidence of the important relationship of ribose nucleic acid in protein synthesis and of the participation of the nucleolus-associated chromatin, the nucleolus and the nuclear membrane is based on many

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From the Institute for Cell Research, Karolinska Institutet, Stockholm, Sweden, and the Department of Pathology, Washington University School of Medicine, St. Louis.

1. (*a*) Caspersson, T.: Symp., Soc. Exper. Biol., no. 1, 1947, p. 127. (*b*) Darlington, C. D.: Nature, London **149**:66, 1942; (*c*) Symp., Soc. Exper. Biol., no. 1, 1947, p. 252. (*d*) Koller, P. C.: ibid., 1947, p. 270. (*e*) Schultz, J.; Caspersson, T., and Aquilonius, L.: Proc. Nat. Acad. Sc. **26**:515, 1940.

2. Stowell, R. E.: Am. J. Path. **23**:883, 1947.

3. (*a*) Caspersson.[1a] (*b*) Hydén, H.: Symp., Soc. Exper. Biol., no. 1, 1947, p. 152. (*c*) Thorell, B.: Acta med. Scandinav. **117**:334, 1947. (*d*) Caspersson, T., and Santesson, L.: Acta radiol., 1942, supp. 46.

observations on cells of mammals, birds, fish, insects, plants, bacteria and yeasts. The cells represent a wide variety of normal and pathologic conditions of retarded and stimulated growth, including adult, embryonic and neoplastic tissues. However, relatively few observations have been made with these technics on regenerating tissues.

The morphologic changes during liver regeneration have been studied and reviewed by numerous investigators,[4] who have shown that the liver is capable of replacing two thirds of its volume within a few days after partial hepatectomy. Among the factors that influence the rate of increase of liver volume are species, diet, age, blood flow and degree of partial hepatectomy. Since the liver substance is restored by diffuse hypertrophy and hyperplasia of the remaining parenchymal cells rather than by regrowth from the site of extirpation, from the point of view of experimental morphogenesis the appropriateness of the term "regeneration" may be questioned (Fishback [4c]; Higgins and Anderson [4d]; Sulkin [4e]). However, the term "regeneration" is used more broadly in medicine, and the widely accepted usage of "liver regeneration" contraindicates an attempt to introduce a more precise term, with the inevitable confusion attending it.

Few previous investigators have made quantitative measurements on changes in cellular constituents in the early stages of liver regeneration. Sulkin [4e] made quantitative measurements of nuclear size after 28 days of regeneration. By this time the liver has almost returned to its normal condition, and one could not expect to learn much of the initial mechanism of the restoration processes. He did observe a mean increase of about 5 per cent in the nuclear diameters of the regenerating cells, twice as many binucleate cells and a greater frequency of polyploidy.

Brues, Drury and Brues [4a] made counts of the cells of liver at various stages of regeneration and found virtually no increase in number during the first day, while there was a 50 to 60 per cent increase in size. During the second day the weight of the liver increased 44 per cent and the number of cells 64 per cent. On the third day the weight increased less than 10 per cent and the number of cells by 26 per cent. They observed that the mean cell size decreased after cell division was initiated but that the size remained a little larger than the normal for twelve days.

Ferreira [4b] measured nuclear and nucleolar areas in regenerating and normal rat liver and found that they were largest at the second day. Only 20 cells were measured in a rat at each stage of regeneration.

4. (*a*) Brues, A. M.; Drury, D. R., and Brues, M. C.: Arch. Path. **22**:658, 1936. (*b*) Ferreira, A. E. M.: Folia anat. micr. conimb. **15**:1, 1940. (*c*) Fishback, F. C.: Arch. Path. **7**:955, 1929. (*d*) Higgins, G. M., and Anderson, R. M.: ibid. **12**:186, 1931. (*e*) Sulkin, N. M.: Am. J. Anat. **73**:107, 1943.

The initial rapid increase in liver substance is comparable to the enlargement of a developing 8 to 10 day chick embryo [5] or to that of a proliferating tissue culture [6] and greater than that of most cancers.[7] Such rapidly growing liver tissue should be especially suitable for correlated studies of morphologic changes in cells and in their chemical constituents, including the nucleic acids.

MATERIALS AND METHODS

Young adult white and piebald rats of unselected lineage weighing 200 to 350 Gm. were maintained on a synthetic diet to facilitate comparison of these results with those of other experiments. The composition of the diet was 45 per cent wheat starch, 14 per cent sucrose, 11.5 per cent casein, 0.5 per cent l-cystine, 4 per cent Osborne-Mendel inorganic salt mixture, 5 per cent brewers' yeast, 19 per cent hydrogenated soy bean oil and 1 per cent cod liver oil. To remove the more labile hepatic cellular substances influenced by diet, the rats were initially fasted 20 hours preoperatively and again as nearly a comparable time as the conditions of the experiment would permit before the animal was killed to obtain the regenerating liver. Drinking water was freely available.

With the rat under ether anesthesia, the median and left lateral lobes of the liver were removed according to the technic described by Brues, Drury and Brues.[4a] Preliminary experiments indicated that 65 per cent of the total liver substance was extirpated by this method. The rats responded well to the operation, so that secondary effects on the liver were considered minimal.

Preliminary observations on regeneration extending to 15 days showed the maximum changes of nucleolar volume at 24 hours. Therefore the changes in regeneration during the first 48 hours were carefully studied at six hour intervals on a total of 13 rats. The early stages of liver regeneration have received relatively little attention from most other investigators.

Liver tissue obtained from each rat at the time of partial hepatectomy and after a determined postoperative period of regeneration was preserved by the Altmann-Gersh [8] freezing-drying technic and by fixation in Stieve, Carnoy, formaldehyde and Regaud solutions, absolute alcohol and cold acetone. Thus all measurements on regenerating liver could be referred to the normal liver of the same animal and a variety of fixed tissues after comparable treatment were available for special or comparative purposes. Fixation in Stieve fluid, consisting of saturated aqueous mercuric chloride 76 cc., formaldehyde solution U. S. P. 20 cc. and glacial acetic acid 4 cc., for 18 hours was followed by washing in 95 per cent alcohol. Dehydrated tissues were infiltrated with paraffin and sectioned usually at 4 microns thickness.

Morphologic measurements of ratios of tissue space, cytoplasm and nucleus were made by Chalkley's [9] method for recording ratios of points indicated by ocular pointers. This method is a relatively rapid and accurate means of determining

5. Carrel, A., and Ebeling, A. H.: J. Exper. Med. **48**:105, 1928.
6. Murray, H. A.: J. Gen. Physiol. **9**:29, 1926.
7. Bashford, E. F.: Scient. Rep. Imp. Cancer Research Fund **4**:197, 1911.
8. Gersh, I.: Anat. Rec. **53**:309, 1932.
9. Chalkley, H. W.: J. Nat. Cancer Inst. **4**:47, 1943.

ratios of different morphologic tissue constituents. For each tissue a total of 800 loci were recorded on a differential blood-counting machine, which is efficiently adaptable for this purpose.

Nuclear and nucleolar diameters were measured in the same plane on 100 hepatic parenchymal cells with an eye piece screw micrometer of the filar type on sections stained by the Feulgen reaction[10] and a light green counterstain. Since the nucleolus is surrounded with chromatin which stains by the Feulgen reaction for thymonucleic acid, the diameter of nucleolar material between and not including the nucleolar associated chromatin was measured. By assuming that the structures were spherical the respective nuclear and nucleolar volumes were calculated from the formula $4/3\ \pi\ r^3$. The errors arising from slight deviations from a true spherical shape and from observation in less than maximum diameter were not considered significant, since they are largely obviated by averaging 100 measurements and by using results only for comparative purposes. From these data the mean relative cytoplasmic volume can be calculated by multiplying the mean nuclear volume by the percentage cytoplasmic volume and dividing by the percentage nuclear volume as determined by Chalkley's ratio measurements. Thus relative morphologic data on nucleolar, nuclear, cytoplasmic and cell volume were obtained for each corresponding normal and regenerating liver, and the significance of the results was analyzed statistically.

Absorption curves of small areas of the cytoplasm of hepatic cells were made according to the ultramicrospectrophotometric method developed by Caspersson.[11] The absorption of ultraviolet rays at wavelengths ranging from 240 to 350 millimicrons was determined photometrically by measuring and comparing (1) the transmission of monochromatic light passing through a predetermined part of the cell mounted on a special quartz microscope and (2) the blank light transmission adjacent to the tissue. From the calculated extinction coefficients the absorption curves can be computed. A correction for nonspecific light scattering and loss in the tissues can be applied according to Rawleigh's formula. Examples of such spectrophotometric absorption curves are shown in figure 1. In this experiment large numbers of such determinations were not made, so that it was not possible to establish the presence of significant differences in the cytoplasm of normal and regenerating cells by this method. Purines and pyrimidines have a high absorption in the region of 260 millimicrons. Because of their predominant distribution in nucleotides, under the conditions of measurement, the absorption maximum at 260 corresponds well to the concentration of nucleic acids. Proteins have a much less pronounced and less precise absorption in the region of 280 millimicrons. Thus under properly controlled conditions one can study the distribution and concentration of nucleic acids and proteins within cellular parts with an area of 1 square micron. Details of the methods and the apparatus and their accuracy have been published.[11]

After the ultraviolet ray absorption characteristics of a tissue have been established by the direct photoelectric technic, additional absorption data can be obtained if necessary by a photographic method.[3d] Tissue sections of 4 microns' thickness mounted on quartz slides in glycerin (specific gravity, 1.25) were photographed with monochromatic light under carefully controlled conditions.

10. Stowell, R. E.: Stain Technol. **21**:137, 1946.

11. Caspersson, T.: (*a*) Skandinav. Arch. f. Physiol., vol. 73, supp. 8; J. Roy. Micr. Soc. **60**:8, 1940; (*b*) footnote 1*a*.

The specimens were placed on a quartz microscope employing Zeiss monochromatic lenses corrected for 257 or 275 millimicrons. From a Köhler rotating cadmium spark gap light source monochromatic illumination of 231, 257 or 275 millimicrons was used. By means of a Köhler view finder, which provides a magnification of the image focused on a fluorescent screen, one can select areas suitable for photography and achieve precise focusing. The same area was photographed for comparative absorption measurements at the different wavelengths.

Although numerous precautions were taken to control variable factors in exposing and developing the photographic plates, a densitometric calibration was made on each plate. On a free space adjacent to the image of the tissue photographed, the image of a rotating step sector was recorded. This provided values for 10, 20, 40, 60, 80 and 100 per cent transmission which could be used in calibrating the optical densities of the tissue image. Densities of the photographic plate for the calibration and comparable cell images on corresponding plates were

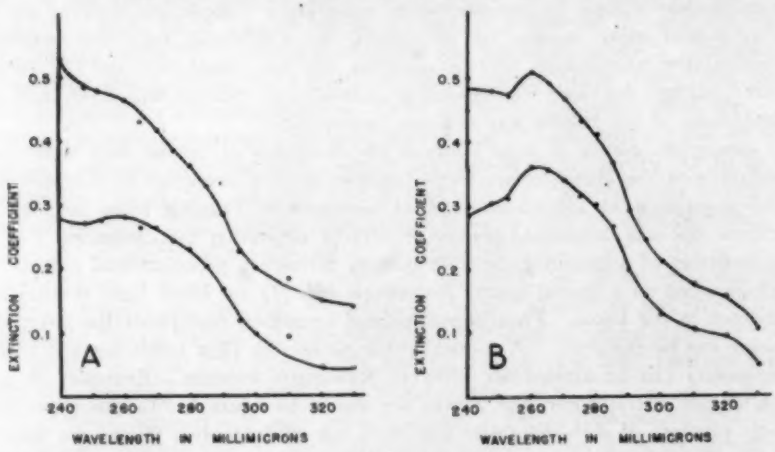

Fig. 1.—Absorption curves of cytoplasm of normal (*A*) and regenerating (*B*) liver cells. The lower curve has been corrected for light scattering and dispersion.

determined with automatic recording microdensitometers of the Leitz or Knorr-Albers type.[12] Light transmission through areas of 0.5 by 1 mm. of the plate was recorded by the densitometer.

By making a continuous densitometric recording across the nucleus and cytoplasm of a cell one obtained a curve as shown in figure 2. Here one can see the correspondence between the high density of such cellular constituents as nuclear membrane and nucleolus and the transmission peaks of the recording. Generally, most normal and regenerating liver cells showed increasing cytoplasmic absorption as one approached the nuclear membrane. Percentage transmission readings were obtained for photographs of the same cells taken at 257 and 275 millimicrons for two corresponding cytoplasmic points taken near but not at the nuclear membrane and for the absorption maximum for the nucleolus. From the corrected transmission readings the optical density or extinction coefficients were calculated for

12. The Aluminum Ore Company, East St. Louis, Ill., allowed us to use their instrument in making many of these determinations.

each wavelength. Duplicate determinations on the same cell in different photographs showed good agreement of densitometric tracings, although the over-all accuracy of this photographic technic is less quantitative than the direct photoelectric method. In instances in which there was considerable difference in the focus of the photographs at 257 and 275 millimicrons, it was found advisable to discard the plates to avoid errors in the results.

RESULTS

Following removal of two thirds of the liver there is a rapid increase in the weight of the remaining tissue, so that in these experiments the original weight was virtually regained by the third day. Examination of sections of normal and regenerating liver from the same animal at six hour intervals showed a decrease in the vascular

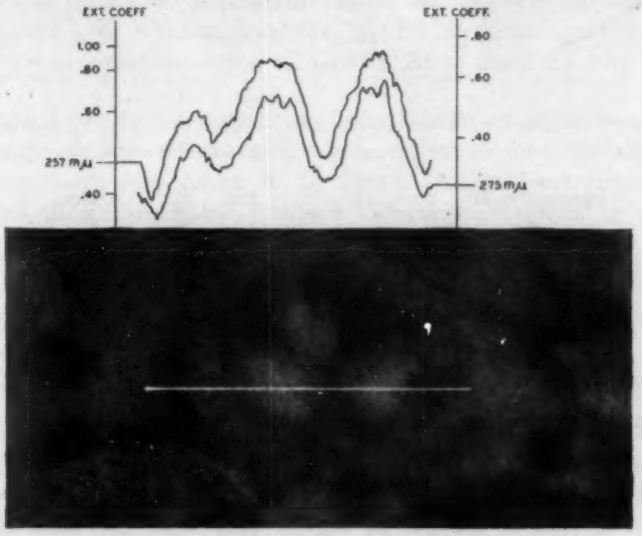

Fig. 2.—Densitometric tracings of photographs taken at 257 and 275 millimicrons wavelength. The line on the photograph of the liver cell shows the path of the densitometric recording across cytoplasm, nuclear membrane and two nucleoli. The correspondence of absorption with cell structures is evident, especially at the nuclear membrane and the nucleoli.

spaces and an increase in the size of the regenerating hepatic cells. The cytoplasm of the cells containing an increased amount of vacuolation, which was demonstrated by sudan staining to be chiefly fat. As indicated in table 1, this change was quite evident by 6 hours and was most pronouced at 18 to 24 hours. Mitotic figures were seen at many stages but in these experiments were most frequent at 30 hours.

The measurements of ratios of tissue constituents by Chalkley's method showed that the regenerating liver had percentages of vascular tissues amounting to 31 to 47 per cent of that of the normal. Vascular tissue. which included the vessel wall, the Kupffer cells and the lumen,

comprised an average of 28 per cent of normal liver substance in these animals. Normal rat liver cells were 91 per cent by volume cytoplasm. Since the nucleoli comprised less than 1 per cent of the normal cell volume, it was more accurate to compute their volume on the basis of their diameters. Comparison of nucleolar and nuclear volumes computed from diameters indicates that the nucleoli of normal rat liver cells constitute 0.4 to 0.7 per cent of the nuclear volume. The results of the volumetric measurements are shown in table 2 and figure 3. The values for P in table 2 indicate the probability of obtaining comparable results by chance. Values less than 0.01, which signify that there is 1 chance in 100 of getting the same results by accident, are considered statistically significant. Thus even after 6 to 12 hours of regeneration significant increases in the mean volumes of cytoplasm, nucleus and nucleolus have occurred. Their volumes increase to a maximum of 2.6, 2.2 and 4.1 times at 18, 24 and 24 hours, respectively. Following

TABLE 1.—*Changes Noted in the Early Stages of Liver Regeneration*

Period of Regeneration, Hr.	Wt. of Liver, Percentage	Fat	Mitosis
6	..	++	0
12	50	++	0
18	..	+++	0
24	55	+++	+
30	..	++	+++
36	65	++	++
42	..	++	+
48	85	++	+
72	100	+	0

the increased cell division which begins at 24 hours and reaches a maximum at 30 to 36 hours, the volumes of the regenerating cell and its constituents decrease toward normal. The nucleus shows more pronounced volume changes in regeneration than the nucleus or the cytoplasm. Observations on young rats, in which regeneration proceeds more rapidly,[13] indicated increase in nucleolar volume as high as 7.5 times at 24 hours.

The mean coefficient of variation of the normal nuclei was 37 per cent and that of the regenerating nuclei 39 per cent. The nucleolar size during the second day showed more variation than in normal cells, the coefficients of variation being 73 and 94 per cent, respectively.

The number of nucleoli per hundred nuclei were counted. It is difficult to express these results in terms of the complete nucleus since, because of its size, usually all of a nucleus was not included in a 4 micron section. There was an average of 1.4 nucleoli per nuclear section in the normal liver. As shown in figure 3, definite fluctuations

13. Norris, J. L.; Blanchard, J., and Povolny, C.: Arch. Path. **34**:208, 1942.

were observed in regenerating cells in the number of nucleoli per nuclear section, in the mean nucleolar mass and in the mean nucleolar mass per nuclear section. The observed changes would not seem to be adequately explained by the possibility that sections of larger nuclei

TABLE 2.—*Mean Morphologic Changes Noted in the Volumes of the Cytoplasm, the Nucleus and the Nucleolus of Regenerating Liver Cells as Compared with Normal Cells of the Same Rat, Expressed as Percentage Increase*

Period of Regeneration, Hr.	Cytoplasm	Nucleus	P	Nucleolus	P
6	144	120	0.0007	120	0.0168
12	122	122	0.0000	219	0.0000
18	236	140	0.0000	288	0.0000
24	239	217	0.0000	406	0.0000
30	236	156	0.0694	206	0.0000
36	164	133	0.0000	102	0.0004
42	187	134	0.0018	133	0.0882
48	143	147	0.0000	74	0.0008

would show a smaller proportion of the total number of nucleoli within the complete nucleus. Generally, when nucleoli were observed to be larger, they were also found to be fewer. This is corroborated by the results shown in figure 3, where diverse fluctuations in number and size of nucleoli are evident.

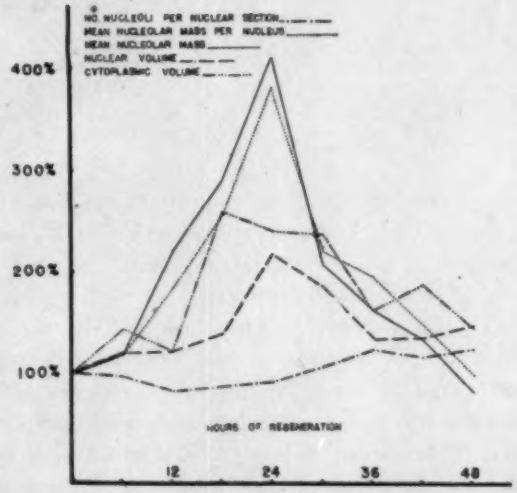

Fig. 3.—Volume of cellular constituents during liver regeneration as compared with normal.

Comparison of ultraviolet photographs of normal and regenerating liver (fig. 4) showed morphologic changes, such as increased fat vacuolation of cytoplasm and increased size of nuclei and nucleoli. Furthermore, these photographs showed an augmented cytoplasmic absorption of ultraviolet rays, especially noticeable adjacent to the nuclear membrane.

This presumptive evidence of increased nucleic acid in regeneration is confirmed by the quantitative cytochemical and macrochemical data to be mentioned. Although these sections are unstained and relatively unfixed by the freezing-drying process employed, they provide a picture in utraviolet absorption similar to that seen with stained fixed sections in visible light. Comparison of ultraviolet photographs of such frozen-dried tissue and tissues fixed in Carnoy or in Stieve fluid showed them to be quite similar.

The results of densitometric measurements of ultraviolet photographs of frozen-dried normal and regenerating cells are given in table 3. The values for normal tissue included measurements from three different specimens; those for 36 hours regeneration were from 2 rat livers, and all others from 1 liver. Sometimes more than one nucleolus was measured in the same nucleus. The direct comparison of measurements of extinction coefficients of cells in different sections is difficult because

TABLE 3.—*Mean Ratios of Absorption Measurements of Wavelengths of 257 to 275 Millimicrons and of Protein and Nucleic Acid in Nucleoli and Cytoplasm of Regenerating Liver Cells*

Period of Regeneraiton, Hr.	Cells	Nucleolus		Cytoplasm	
		E257/E275	P/NA	E257/E275	P/NA
0	23	1.16	34	1.09	45
12	4	1.12	40	1.07	48
24	39	1.18	32	1.11	42
30	12	1.21	27	1.11	42
36	9	1.30	18	1.13	38
42	4	1.40	13	1.27	20

slight variation of section thickness can produce considerable changes in ultraviolet absorption. Therefore, when one is not comparing parts of the same section, it is often better to relate the absorption at 257 millimicrons, which is markedly affected by nucleic acids, to that at 275 millimicrons, which, predominantly produced by proteins, is much less readily affected in changes of the concentration of protein or nucleic acid. The use of ratios, furthermore, reduces errors from the inability to correct for light scattering and dispersion in the photographed tissues. The mean ratios of extinction coefficients of 257 to 275 millimicrons shown in table 3 indicate that there is an increase during regeneration in the total nucleic acid of the nucleoli and of the juxtanuclear cytoplasm. Statistical analysis showed that this increase over normal is significant at 42 hours for both nucleolus and cytoplasm and at 36 hours for the nucleolus. The ratios of protein (P) to nucleic acid (NA) are taken from data compiled from absorption measurements of a standard protein by Caspersson and Santesson.[14]

14. Caspersson and Santesson,[3d] p. 38.

To check macrochemically the ultraviolet cytochemical measurements, Prof. E. Hammarsten [15] made determinations of total phosphorus and of percentage ribonucleotides of all nucleotides on several control and regenerating liver specimens from these same animals. The

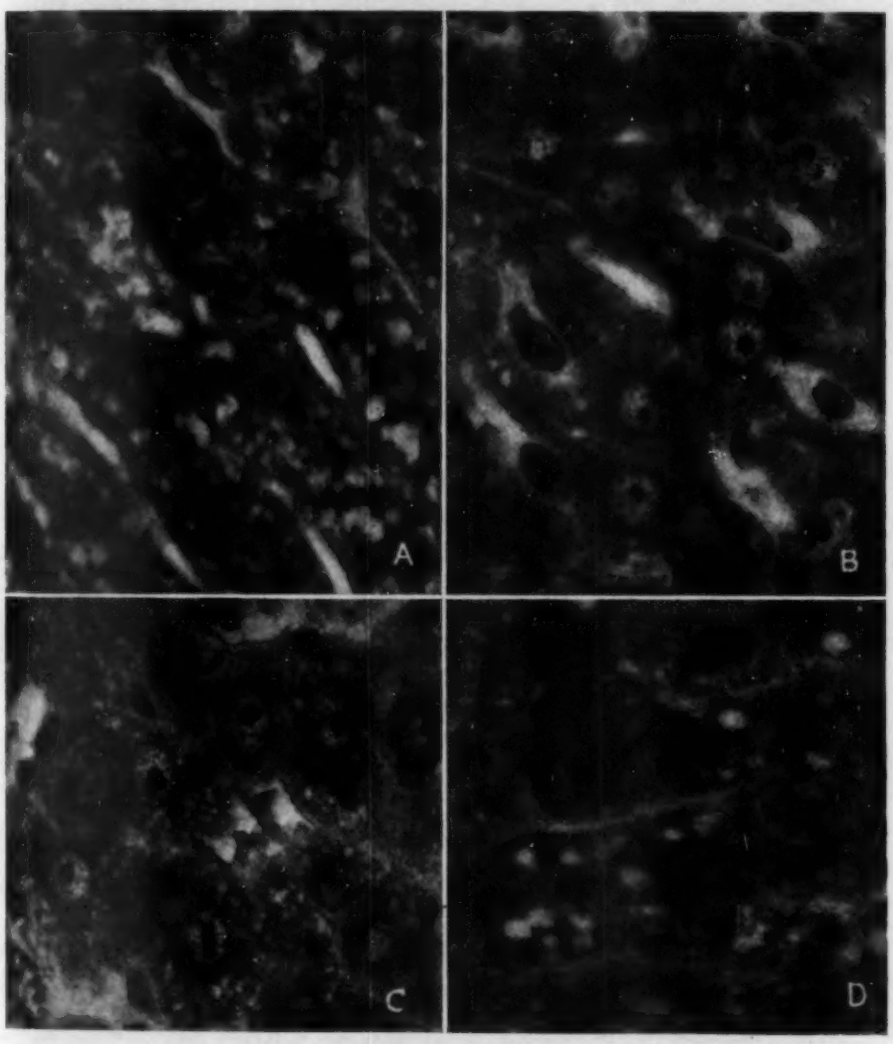

Fig.4.—Sections of unstained rat liver all photographed at 257 millimicrons wavelength; × 1,000.

A, high juxtanuclear absorption and vacuolated cytoplasm seen in this area at 24 hours' regeneration; tissue fixed in Stieve solution.

B, normal liver; frozen, dried tissue.

C, 24 hours' regeneration; frozen, dried tissue.

D, 36 hours' regeneration; frozen, dried tissue.

15. Hammarsten, E.: Personal communication of results, with methods to be published.

results in table 4 indicate an increase in total nucleic acid as well as in the ribose fractions during liver regeneration. With the technic employed, the low nucleotides were less than 10 per cent of the total nucleotides.

COMMENT

A comparison of the morphologic and cytochemical measurements indicates interesting relationships. Within the first six hours of regeneration the cells showed a significant increase in the volumes of cytoplasm, nucleus and nucleolus and a decrease in vascular space. The increased visible cytoplasmic lipids suggest altered cellular metabolism. The earliest swelling of the hepatic cells may be associated with increase of lipids and imbibition of fluids rather than being primarily accounted for by increased protein synthesis. Although the volume of the total liver, as well as that of the cells, increased considerably, relatively few cells were dividing during the first day. By 24 hours the mean cyto-

TABLE 4.—*Macrochemical Determination of Percentage of Total Phosphorus and of Percentage of All Nucleotides That Were Ribose Type in Regenerating Liver Cells (Hammarsten [15])*

Stage of Regeneration Represented in Specimen	Total Phosphorus, Percentage	Ribose, Percentage
Control	0.43	64
12 hr.	0.44	70
24 hr.	0.53	79
42 hr.	0.56	70

plasmic, nuclear and nucleolar volumes had increased 2.4, 2.2 and 4.1 times, respectively; yet the absorption coefficient measurements suggest no appreciable change in percentage composition of nucleic acid and protein in either nucleolus or cytoplasm. The incidence of cell division increased greatly at 30 hours and was accompanied, as might be expected, by some decrease in mean volume of cytoplasm, nucleus and nucleolus. Marshak and Byron [16] found that the percentage of cells in mitosis increased from 0.03 to 0.37, 0.47 and 0.27 on the first, second and third day of regeneration. Brues and Marble [17] reported an increase from less than 0.001 per cent mitoses to 2.1 per cent at 24 hours with a decline toward normal during the next two days. Thus, all cells show an initial tremendous increase of their constituents, but after they start dividing, their mean volume decreases. With the augmented mitotic activity and associated production of cellular constituents there is good cytochemical evidence of an increase in the concentration of nucleic acids in the cytoplasm near the nuclear membrane. Although

16. Marshak, A., and Byron, R. L.: Proc. Soc. Exper. Biol. & Med. **59**:200, 1945.

17. Brues, A. M., and Marble, B. B.: J. Exper. Med. **65**:15, 1937.

the number of cells measured is relatively small at some stages of regeneration, nevertheless the results show a consistent change.

Davidson and Waymouth [18] made macrochemical determinations on nucleic acid in liver under several conditions. In regenerating liver they found that the total nucleoprotein phosphorus was not appreciably altered, the acid-soluble nucleotide concentration was raised and the ratio of ribonucleic acid phosphorus to desoxyribonucleic acid phosphorus was unaltered.

In regenerating as compared with normal rat liver Brues, Drury and Brues [4a] found 19.5 per cent less percentage nitrogen (wet weight) the first day, 18.2 per cent less the second and 7.1 per cent less the third. Although the liver was restored at a somewhat faster rate in this experiment than in theirs, it seems probable that there is increased tissue fluid with little new cellular protein formed in the first day, with considerably more in the second and third days. Measurements of nucleic acids beyond the second day of regeneration might have shown a greater increase in their concentration.

Brues, Tracy and Cohn [19] studied the turnover of radiophosphorus in regenerating and normal liver. Both types of nucleic acid had a greatly increased activity in regeneration, which was attributed to synthesis and turnover.

The interpretation of the chemical changes in the nucleoli is not simple. Although the thickness of the cytoplasm should be similar for comparing photometric measurements of sections of different tissues, in comparing nucleoli one should consider changes in their diameters or volumes. Nucleolar diameters were measured on the photographic plates for those nucleoli on which densitometric determinations were made. Although the computed mean volume of nucleoli measured by this method showed more than twice the normal size at 24 hours, the extinction coefficients for 257 and 275 millimicrons were considerably decreased. Furthermore, the mean extinction coefficients for both wavelengths increased consistently during the subsequent phases of regeneration when mean nucleolar size was decreasing. The evidence from the respective extinction coefficients and their ratios to each other suggests that nucleoli of dividing regenerating cells have a higher concentration of nucleoproteins and especially of the ribose nucleic acid component, which is the principal type found in the nucleolus. The methods employed would, however, measure both the constituents of the nucleolus as well as the overlying nuclear chromatin, which is fortunately scant by comparison with the nucleolar material. Larger nucleoli would more frequently be divided in cutting sections, so that less of

18. Davidson, J. N., and Waymouth, C.: Biochem. J. **38**:379, 1944.
19. Brues, A.; Tracy, M. M., and Cohn, W. E. P.: J. Biol. Chem. **155**:619, 1944.

their total substance would be measured. These sources of error were reduced by utilizing comparative data on numerous cells. Additional experiments on the chemical composition of the nucleolus would be desirable.

Although specimens from each liver were usually fixed and prepared by seven methods, which gave somewhat similar results, the freezing-drying technic was the most generally satisfactory for cytochemical work. It produced less artefact from scattering and dispersion of light by the protoplasmic matrix of cells.

Fortunately, this experiment permits one readily to relate the cytochemical and morphologic data for the regenerating liver to those for the normal liver from the same animal. The expression of results in such relative terms may be much more accurate than that based on the use of more highly quantitative units.

With polyploidy of the liver cells one might expect the increases of chromosomal content to be accompanied by comparable increases of nuclear volume to give groups of nuclei with mean values having ratios of 1:2:4:8:16.

Biesele, Poyner and Painter [20] and Sulkin [40] have computed the volumes of liver cell nuclei by different methods and reported groupings of different-sized nuclei. The first group of investigators made measurements usually on less than 100 nuclei per specimen and found classes with ratios of 1:2.3:4.1. Their class 1 contained a variety of types of cells, including endothelial and blood cells. Sulkin measured 200 nuclei on each of 6 normal and regenerating rat livers and obtained classes with ratios of 1:2.0:4.2. After 28 days of restoration the liver cell nuclei still fell into comparable classes with the same maximum for each mode, but with more of the larger size nuclei.

Beams and King [21] have discussed the relationship of binucleate cells and polyploidy in restoring liver at three days. They found about 20 per cent binucleate cells in both normal and regenerating livers. Plotting of nuclear diameters against their number showed a bimodal curve. The nuclear volumes of the first mode in the second had a ratio of 1:2, with more than twice as many nuclei in the first group of uninuclear cells. Binucleate cells had a similar bimodal curve, with more nuclei in the group with the larger volume. In restoring liver the second mode of the bimodal curves was more scattered, with variable numbers of larger nuclei. For the uninuclear cells the volume of each mode was increased nearly a fourth and the ratio of the first and second modes was about 1:1.7.

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20. Biesele, J. J.; Poyner, H., and Painter, T. S.: Nuclear Phenomena in Mouse Cancers, Publication 4243, University of Texas, 1942.

21. Beams, H. W., and King, R. L.: Anat. Rec. **83**:281, 1942.

Analysis of nuclear volumes on 100 cells in each of the 13 normal livers in this experiment showed that they tended to fall into two distinct classes with a ratio of 1 : 1.75. There was suggestive evidence of the presence of other higher classes, but the number of measurements was inadequate to establish them definitely. There was reasonably close agreement between the classes in different normal livers, the ratios varying from 1 : 1.6 to 1 : 2.1. In regenerating livers such ratios were obtained only at stages under 24 hours. At intervals of 24 to 48 hours there was usually loss of significant modal distribution, even though 500 nuclei were measured on several specimens. This absence of distinct classes may be attributed to the mixture of (1) cells with enlarged nuclei which have not divided with (2) recently divided cells. It is evident that normal liver cell nuclei tend to form several groups related to their chromosome content and that the volumetric relationship of the groups is altered or lost in certain stages of restoration of the liver as it is in many cancers. It is not clear why this study and those of Sulkin [4e] and Biesele, Poyner and Painter [20] found more nuclei in the second class than in the first whereas Beams and King [21] found more in the first in mononuclear cells. The percentage of binucleate cells (20) reported by Beams and King is inadequate to explain this difference.

These experiments, in conjunction with other observations, including those on changes in the nucleoli of the liver cells of rats restricted to low and high protein diets and those made during production of hepatomas with para-dimethylaminoazobenzene,[22] add material support to the evidence for an important relationship between the nucleolus and the cytoplasm in the formation of nucleic acids and proteins. It seems desirable to defer the more theoretic considerations of the relationships of these morphologic and chemical changes in the nucleolus and the cytoplasm until further experimental data are presented.

SUMMARY AND CONCLUSIONS

The changes in cellular structure and nucleic acid concentration were correlated during the first two days of regeneration of rat liver. Within the first six hours of regeneration there was an increase in the visible cytoplasmic lipids and in the volume of the cytoplasm, nucleus and nucleolus with a decrease in the sinusoidal space. By 18 to 24 hours the mean volumes of the cytoplasm, the nucleus and the nucleolus had increased 2.6, 2.2 and 4.1 times, respectively. Following an increase in cell division at 24 to 30 hours, there was a decrease in the mean volume of each of these cellular constituents. At 48 hours' regeneration the mean nuclear and cytoplasmic volumes were still

22. Stowell, R. E.: Cancer, to be published.

considerably above normal, while the mean nucleolar volume was less than normal. In normal livers the nuclear volumes constituted two main groups with mean ratios of approximately 1 to 2. This evidence of normal polyploidy tended to disappear in the second day of regeneration.

Ultraviolet cytochemical observations indicated little change in nucleic acid concentration during the first 24 hours, but during the second day the synthesis associated with the rapid cell division was accompanied by an increase of nucleic acid concentration in the cytoplasm adjacent to the nucleus and also in the nucleolus. The results indicate that the morphologic aspects and the chemical composition of the nucleolus and of the cytoplasm are greatly altered in different phases of growth in regenerating hepatic cells.

University of Kansas.

MITOTIC ACTIVITY IN THE AORTIC LESIONS OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS OF RABBITS

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AND
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THE relatively frequent occurrence of mitotic configurations in the globular foam cells and in the stellate fibroblastic cells of the aortic lesions induced by cholesterol feeding in rabbits appears to have escaped the attention of those interested in the morphologic aspects of experimental cholesterol atherosclerosis. The reviews and reports of such workers as Anitschkow,[1] Leary,[2] Duff[3] and Hueper[4] do not mention mitotic activity. There is general agreement that the fibroblastic elements of the atherosclerotic lesions proliferate, and Hueper[4] stated that vascular endothelial cells multiply and are transformed into globular foam cells. The concept that some, or all, of the lipophages contained in the arterial lesions of experimental cholesterol atherosclerosis arise *in situ* by mitotic division was considered theoretically by Duff,[3] but without the support of morphologic evidence of mitotic activity.

It is our purpose in this report to present evidence that mitotic figures are not uncommon in the cellular components of the intimal lesions of experimental cholesterol atherosclerosis of the aorta.

MATERIALS AND METHODS

The mitoses reported here were found in the atherosclerotic lesions of the aortas of 10 rabbits fed cholesterol as a 5 per cent solution in warm corn oil in a daily dose of about 0.75 Gm. The period of feeding varied from seventy-six to ninety days, and the total dose of cholesterol administered to each animal was from 46 to 65 Gm. On completion of the feeding experiment the animals were killed by air embolism, autopsies made and the opened aortas fixed in 10 to 15 per cent concentration of

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From the Department of Pathology, Pathological Institute, McGill University.

This work was assisted by grants-in-aid from the National Research Council, Canada.

1. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933, chap. 10.

2. Leary, T.: Arch. Path. **17**:453, 1934.

3. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935.

4. Hueper, W. C.: Arch. Path. **38**:162, 245 and 350, 1944; **39**:51, 117 and 187, 1945.

formaldehyde solution U. S. P. in saline solution. After fixation the aorta was rolled into a coil and embedded in paraffin so that a single microscopic section could be made to include the entire length of the organ. Sections 6 microns in thickness were stained with Mallory's phosphotungstic acid-hematoxylin, hematoxylin and eosin or hematein, phloxine and Spanish saffron. The time from death until the aorta was placed in fixative varied from about twenty to ninety minutes. All animals suffered from a relatively severe degree of atherosclerosis of the aorta with extensive confluent intimal plaques measuring up to about one half the thickness of the underlying media.

The criteria of mitotic configuration were those commonly accepted, including the proviso that the phase of mitosis should be clearly recognizable. If a figure was recognized as mitotic but, for technical reasons, the phase of activity was not clearly indicated, then the figure was classified as one of undetermined phase. Configurations that were merely suggestive of mitosis or that represented frank necrobiotic phenomena were excluded.

Summary of Data on Mitotic Activity in Foam Cells and Fibroblastic Cells in Experimental Cholesterol Atherosclerosis of the Aorta

Rabbit	Distribution of Mitoses by Phase				Number in Fibroblasts	Number in Foam Cells	Total Mitoses of Recognized Phase	Mitotic Figures of Undetermined Phase	Total
	Prophase	Metaphase	Anaphase	Telophase					
1	1	2	0	0	0	3	3	4	7
2	3	4	0	0	2	5	7	3	10
3	0	1	0	1	0	2	2	4	6
4	1	1	0	0	0	2	2	2	4
5	0	1	0	0	1	0	1	0	1
6	1	5	1	1	3	8	11	7	18
7	0	0	0	1	0	1	1	1	2
8	0	3	0	1	0	4	4	0	4
9	1	4	0	0	0	5	5	4	9
10	0	4	4	1	2	7	9	7	16
Total	7	23	5	5	8	37	45	32	77

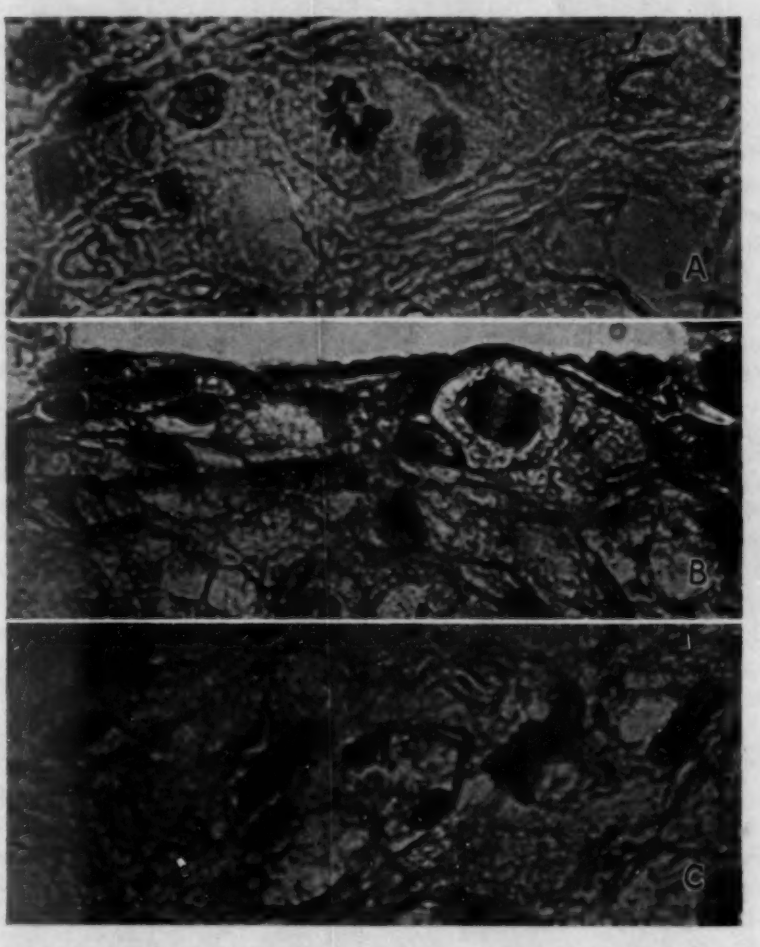
OBSERVATIONS

Among the 10 aortas examined, 6 presented lesions that proved on microscopic examination to consist almost entirely of foam cells. The remaining 4 aortas also presented varying degrees of intimal fibrosis.

A summary of the incidence and phase of the mitotic figures encountered in a single section of the entire length of the aorta of each animal is given in the table. Photomicrographs of some of the different phases of mitotic division in lipophages are shown in the figure.

The table shows that 45 mitotic figures of identifiable phase were observed in the 10 aortas. Of these, 37 were in globular foam cells, and 8 were in fibroblastic cells. Inasmuch as the mitotic figures recorded were encountered in a single section of the entire length of the aorta of each animal, the average number of mitoses seen in the lipophages of each section was 3.7, or about 1 such figure in every 4 cm. of length of the section. In the 4 aortas in which intimal fibrosis also occurred, 8 figures were seen in fibroblastic cells, or about 1 figure in each 7 or 8 cm. of length of the section.

In addition to these 45 mitoses, 32 mitotic figures of uncertain phase of division were observed. If these configurations are also taken into account, an average of 1 mitotic figure was seen in every 2 cm. of length of the sections of aorta 6 microns in thickness. It will be noted that the number of figures seen in each section varied considerably, the least being 1 and the greatest being 18.



Photomicrographs of mitotic figures in foam cells in aortic intimal lesions of experimental cholesterol atherosclerosis: *A*, a mitotic figure in metaphase.
B, a mitotic figure in anaphase.
C, a mitotic figure in telophase.

Mitotic figures were observed in all layers of the atherosclerotic lesions from the most superficial to the deepest, but it was our impression that they were somewhat more frequent in the more superficial cells. Mitotic figures were not associated with signs of degeneration or necro-

sis of adjacent cells. Indeed, in the single aorta in which there was patchy necrosis in the deeper layers of the intimal lesions, mitoses were not observed in relation to these necrotic areas. In addition to the evidence of cellular division, there was evidence of nuclear division alone, demonstrated by the presence of numerous large binucleate foam cells, in the deeper layers of the intimal lesions. In the 10 sections examined, mitotic figures were not observed in the lining endothelial cells.

COMMENT

Before concluding that the mitotic figures observed were truly indicative of cellular division, the possibility was considered that these mitoses might represent necrobiotic or postmortem phenomena. This possibility was excluded by the observation that almost one quarter of the mitotic figures were in typical anaphase or telophase, indicating true mitotic activity, since mitonecrotic division seldom progresses beyond metaphase. Moreover, mitotic figures were not observed to be associated with areas of degeneration or necrosis. The observation of large, well preserved binucleate foam cells in the deeper layers of the atherosclerotic lesions may be interpreted as further evidence that nuclear division is carried to completion in living cells, even though in these particular cells cytoplasmic division apparently failed to occur.

The data presented are inadequate to permit a detailed analysis of the factors concerned in the production of mitotic division in the cellular components of the lesions of experimental cholesterol atherosclerosis of the aorta. Nevertheless, the occurrence and frequency of mitotic division in these cells are evidence that a considerable proportion, if not all, of them arise by local proliferation of preexisting cells of the same types. Accordingly, there remains no compelling reason to assume that the increase in numbers of lipophages in the developing intimal lesions is dependent on a continuous process of migration of such cells into the intima either from the lumen of the artery or from the medial direction. Neither do our observations lend support to the concept that the cellular growth of these lesions depends on repeated mitotic divisions of the lining endothelial cells. Regardless of what theory is proposed relative to the derivation and original source of the foam cells and fibroblasts in the lesions of experimental cholesterol atherosclerosis, it is apparent that a considerable proportion of them arise *in situ* by mitotic division.

SUMMARY

The observed frequent occurrence of mitotic figures in the foam cells and fibroblasts of the aortic intimal lesions of experimental cholesterol atherosclerosis of the rabbit is interpreted as evidence that a considerable proportion, if not all, of these cells arise *in situ* by mitotic division.

Case Reports

DIFFUSE PLASMA CELL MYELOMATOSIS

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THE CONCEPT that plasma cell myeloma is not primarily an osseous lesion but is a diffuse neoplastic disorder of the hemopoietic system has been steadily gaining recognition.[1]

There are numerous cases on record in which one or more of the viscera are involved by myelomatous masses. Diffuse plasma cell involvement of the viscera is infrequent and is usually associated with the appearance of plasma cells in the blood stream. These cases are segregated under the heading of plasma cell leukemia.[1] That diffuse plasma cell myelomatosis may occur without a concomitant leukemic blood picture is shown in the case reports of Lowenhaupt.[2]

Because of the rarity of completely studied cases, an instance of diffuse plasma cell myelomatosis is presented.

REPORT OF A CASE

A 51 year old salesman was admitted to the hospital complaining of pain in the right lateral aspect of his chest. Ten months previously he had profuse and continued rectal bleeding, for which he was given a blood transfusion. Two months previously he first noted loss of appetite and loss of weight, and about this time he noted jaundice, and his liver was found to be enlarged. On admission he stated that he had lost a total of 13.5 Kg. (30 pounds). He had had recent attacks of epistaxis. He was not in the habit of taking alcoholic beverages. Physical examination revealed an obese, well developed, jaundiced man, breathing with difficulty because of thoracic pain. His blood pressure was 98 systolic and 50 diastolic. The heart sounds were rapid, with no murmurs. In the right lower region of the chest anteriorly, dulness, diminished breath sounds and coarse snapping rales were heard. The abdomen was enlarged, with visible veins and ecchymoses. The edge of the liver extended to 10 cm. below the costal margin. He had a left-sided hydrocele. The rectal examination gave negative results.

The significant laboratory data were as follows: red cell count, 1,200,000; hemoglobin content, 26 per cent; white blood cell count, 5,000, with 56 per cent polymorphonuclears, 40 per cent lymphocytes, 4 per cent eosinophils and occasional nucleated red cells. After the autopsy, reexamination of his blood smears revealed 1 to 2 per cent plasma cells. The urine revealed small amounts of albumin but no Bence Jones protein. On admission the icterus index was 50 units; terminally it was 100 units. Serum albumin was 5.3 mg., globulin 1.5 mg. and nonprotein nitrogen 55 to 76 mg. per hundred cubic centimeters. Prothrombin activity was 50 per cent of normal, decreasing to 20 per cent of normal. Aspirated sternal

From the Departments of Pathology and Medicine, University of Vermont College of Medicine.

1. Lubarsch, O.: Virchows Arch. f. path. Anat.: **184**:213, 1906. Jackson, H., Jr.; Parker, F., Jr., and Bethea, J. M.: Am. J. M. Sc. **181**:169, 1931. Piney, A., and Riach, J. S.: Folia haemat. **46**:37, 1931. Moss, W. T., and Ackerman, L. V.: Blood. **1**:396, 1946.

2. Lowenhaupt, E.: Am. J. Path. **21**:171, 1945.

marrow revealed a preponderance of plasma cells, which were in various stages of development, with many irregular forms (fig. 1).

Roentgenograms revealed widespread small osteolytic lesions of the ribs, the scapulas and to a lesser extent of the spinal column and the pelvis. A barium sulfate enema and a gastrointestinal series showed no abnormality.

The patient's course was steadily downhill, with increasing weakness, deepening jaundice and terminally many petechial hemorrhages. His temperature remained at about 101 F. His spleen became palpable after the second week of hospitalization. He died on the twenty-eighth hospital day.

Autopsy.—The body was that of a white man and weighed 84 Kg. (185 pounds) and measured 180 cm. The skin and the scleras were deeply icteric. Numerous petechial hemorrhages were noted on the arms, the thighs and the legs. The superficial lymph nodes were not enlarged. There was a recent biopsy incision over the tip of the right scapula.

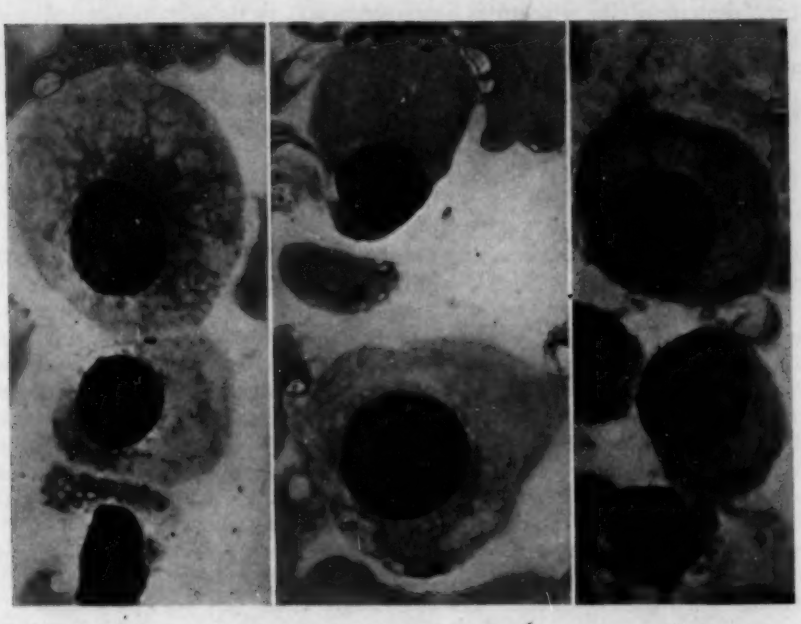

Fig. 1.—Plasma cells in bone marrow smears, illustrating various stages of development and irregular forms; × 1,000; Wright stain.

The peritoneal cavity contained approximately 600 cc. of clear icteric fluid. The liver's edge extended 12 cm. below the costal margin in the right midclavicular line. Along the common bile duct there was a chain of lymph nodes which were firm and measured 2 to 3 cm. in diameter. The mesenteric and some of the retroperitoneal nodes were slightly enlarged.

The pleural cavity contained about 50 cc. of clear fluid on each side. There were fractures of the first, third and sixth ribs on the left side and the third and sixth ribs on the right side. At these sites palpable nodular enlargements with subpleural ecchymoses were found.

The mediastinal nodes were not enlarged.

The heart was not remarkable.

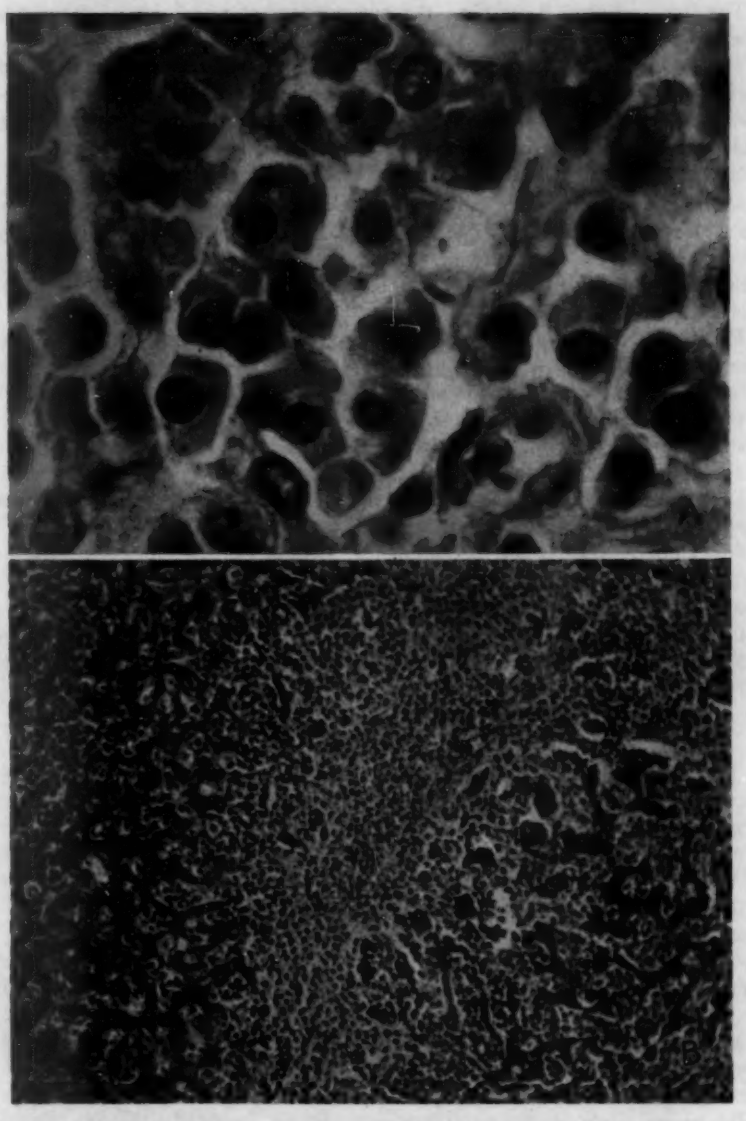

Fig. 2.—*A*, lymph node showing crowding of the sinusoids with plasma cells; × 1,000; hematoxylin and eosin. *B*, liver showing extensive plasma cell infiltration of hepatic sinuses and periportal connective tissue; × 100; hematoxylin and eosin.

The lungs showed severe congestion.

The spleen weighed 730 Gm., and the cut sections were homogeneously gray-red and moderately firm.

The liver weighed 3,400 Gm., the cut surfaces of which revealed the lobular structure accentuated by a pale gray markings. The biliary tract was patent.

The marrow of the vertebrae, the sternum and the ribs was replaced by diffuse grayish soft tissue. An incidental finding was an 8 cm. renal tumor which was typical of renal cell carcinoma ("hypernephroma") both grossly and microscopically.

No gross myelomatous nodules were found in any of the viscera.

Microscopic Examination.—The spleen revealed complete alteration of structure with absence of follicles and diffuse plasma cell infiltration in various stages of development. While many of the larger cells had nuclear and cytoplasmic characteristics akin to those of reticulum cells, others had the distinctive features of well differentiated plasma cells.

The lymph nodes showed even more complete plasma cell replacement of all elements (fig. 2 *A*).

In the liver, cellular infiltration was most striking, being diffuse throughout the parenchyma and especially dense in the peripheral zones of the lobules (fig. 2 *B*). This was associated with severe retrogressive changes of the hepatic cords. Bile casts were present in many of the ductules.

The lungs showed severe interstitial pneumonia, in connection with which numerous plasma cells were found together with other mononuclear cells.

The kidneys, aside from the carcinoma, showed normal glomeruli, but severe tubular degeneration was evident, with fibrosis of the interstitial tissue and occasional focal collection of plasma cells.

The marrow of the sternum, the ribs and the vertebrae revealed diffuse plasmacytic infiltration. These cells were found freely intermingled with the other marrow elements rather than as compact "plasmacytomas."

COMMENT

The anatomic distribution of plasma cells illustrated in the case described differs in no essential feature from that observed in other forms of leukemia with the exception that there was no invasion of the blood stream. For this reason the term "plasma cell myelosis" or "plasma cell myelomatosis" may be applied in such cases. To refer to the cell type as "myeloma cell," as has been the tendency in the recent literature,[3] does not seem any more reasonable than to call the abnormal lymphoid cells appearing in lymphatic leukemia by a special name. The cells of myelomatosis can usually be identified as belonging to the plasma cell series, and various developmental stages may be observed (fig. 1). Admittedly, many bizarre forms may occur in varying proportion, just as in other forms of leukosis. The "typical" cart wheel nucleus of the plasma cell as seen in tissue sections is practically never seen in marrow smears and is probably due to fixation.

SUMMARY

A case is presented in which at autopsy a white man was revealed to have diffuse plasma cell infiltration of the viscera of a type usually seen in plasma cell leukemia, but in this instance without a significant number of plasma cells in the blood stream.

3. Winthrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1946.

ADENOMA OF THE PAROTID GLAND

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ACKERMAN,[1] in 1943, reviewed the literature, tabulated 7 cases which he felt were authentic and added a new case. Salivary gland adenoma is also termed oncocytoma, since it supposedly arises from oncocytes. Stout [2] recently quoted the work of Hamperl, who originated the term "oncocyte" and listed the various organs in which this type of cell is found. Oncocytes of the salivary glands reportedly take origin from the tubules and terminal secreting portions of seemingly normal tissue.[3] Ackerman and Regato [4] stated that oncocytoma is an adenoma and that probably in many instances it arises from duct epithelium. Gruenfeld,[3] in reporting a case of oncocytoma, stated that the histologic picture suggested an origin from the duct system. Schutz,[5] in quoting Lambret, mentioned that salivary adenoma is of two types and originates from either duct epithelium or acinous epithelium. Gruenfeld [3] stated that Huckel and Franssen described parathyroid-like new growths of the parotid gland which they believed to be adenoma developing from the parenchymal cells.

The case to be reported is one of adenoma of the parotid gland, but it differs from the reported cases in that the tumor apparently arose from acinous epithelium.

REPORT OF CASE

H. S., a 38 year old white woman, complained of swelling of the left side of the face, which was first noticed two years previously. The mass steadily increased in size. At no time was it painful, although a stinging sensation occasionally occurred, lasting for a few seconds. The lesion was soft and small in the morning but by late afternoon was large and firm. Located at the angle of the left mandible was a well defined mass about 2.5 cm. in diameter which was not tender and not attached to the overlying skin.

At operation a well encapsulated soft tumor was found within the parotid parenchyma. It was completely removed with a minimal amount of the surrounding

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From the Department of Pathology, Touro Infirmary.

1. Ackerman, L. V.: Arch. Path. **36**:508, 1943.
2. Stout, A. P.: Arch. Path. **35**:803, 1943.
3. Gruenfeld, G. E., and Jorstad, L. H.: Am. J. Cancer **26**:571, 1936.
4. Ackerman, L. V., and del Regato, J. A.: Cancer Diagnosis, Treatment and Prognosis, St. Louis, C. V. Mosby Company, 1947.
5. Schutz, C. B.: Am. J. Path. **2**:153, 1926.

parotid gland. In removal the capsule was ruptured, with liberation of brown mucoid material. The specimen was placed in 4 per cent formaldehyde solution, sectioned and stained with hematoxylin and eosin, Mayer's mucicarmine, Mallory's aniline blue, Masson's trichrome stain and sudan IV.

Grossly the tumor measured about 4 cm. in diameter. The tissue was soft and appeared homogeneously pinkish gray. The capsule was complete except for the point of surgical rupture.

Microscopic sections showed the tumor to be completely encapsulated by fibrous tissue and surrounded by small areas of normal-appearing salivary gland. There was a single lymph node external to the capsule. In the capsule were a few focal collections of lymphocytes. The cells composing the neoplasm were arranged in sheets with occasional acinus formation, which was most noticeable at the periphery. The cells were better stained in the peripheral portions of the tumor, where they showed a well defined cell wall and an eccentric nucleus. The cytoplasm varied

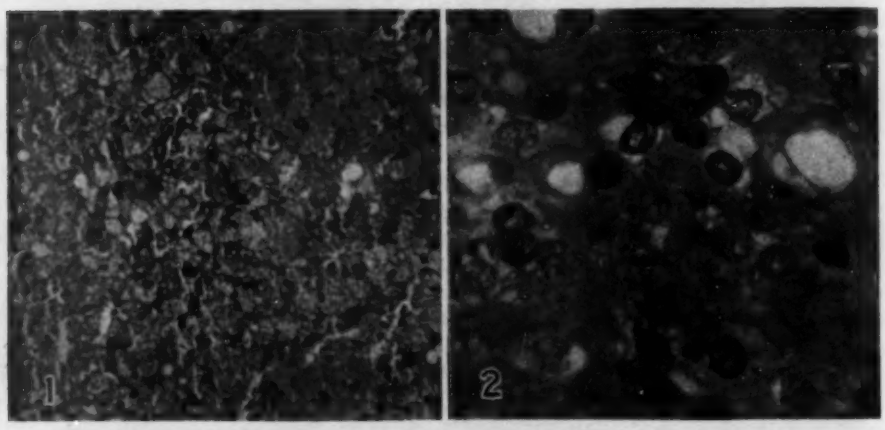

Fig. 1.—Adenoma of a parotid gland; hematoxylin and eosin; × 90.

Fig. 2.—Section of the tumor, showing vacuolation and the nuclear and cytoplasmic structure; hematoxylin and eosin stain; × 450.

from finely granular to vacuolated. The granular cells composed the major portion of the tissue, and their cytoplasm took a pale eosinophilic stain. The nuclei were predominantly round or oval, light staining and finely stippled. A few were small and stained dark blue. Between the cells were many vacuoles which resembled the sites of secreted material. Many of the vacuolated cells appeared to have ruptured with extravasation of their material. In some areas there were wide zones of vacuolated homogeneous light pink–staining material resembling colloid. No mitotic activity was observed. The vascularity was not pronounced, and the stroma was sparse, there being only occasional strands of fibrous tissue scattered through the tumor. No ductal structures were demonstrated. An occasional small focal accumulation of lymphocytes was present. The mucicarmine stain gave negative results, and sudan IV revealed finely divided interstitial fatty particles. The vacuolated areas revealed nothing with sudan IV. Masson's stain did not demonstrate eosinophilic granules. Mallory's stain revealed fine dark blue cytoplasmic granules, which were similar to those seen in the acinous cells of normal glands with control stains.

COMMENT

In the few reported cases [1] salivary gland adenoma occurred more commonly in women around the sixth decade and had a duration of a few months to several years. It attained a size of several centimeters, was usually firm, gray to brownish red and not attached to the skin. It had a characteristic microscopic picture.

In this case the tumor occurred in a 38 year old white woman. It was completely encapsulated. The tissue showed intercellular and intracellular vacuolation, intercellular colloid-like material and fine blue cytoplasmic granules. These three features suggest secretory activity and may explain the daily swelling of the tumor. No salivary ducts were found to suggest an origin from these elements, and none of the cells resembled the markedly eosinophilic cells termed oncocytes. The cells did not stain with sudan IV [6] or with Masson's [2] trichrome stain as has been reported in regard to oncocytes. There has been no recurrence of the tumor after six months.

SUMMARY

Herein is reported an unusual case of adenoma of the parotid gland. It differs from the reported cases of adenoma (oncocytoma) in that the patient was younger, encapsulation of the growth was complete, acini were present and there was evidence of secretory activity. The individual cells resembled the acinous elements rather than the large granular eosinophilic cells seen in oncocytoma.

It is therefore believed that the reported adenoma arose from acinous epithelium in contradistinction to oncocytoma, which probably has a ductal origin.

6. Jaffé, R. H.: Am. J. Cancer **16**:1415, 1932.

Books Received

EDUCATION FOR PROFESSIONAL RESPONSIBILITY: A REPORT OF THE PROCEEDINGS OF THE "INTER-PROFESSIONS CONFERENCE ON EDUCATION FOR PROFESSIONAL RESPONSIBILITY" HELD AT BUCK HILL FALLS, PENNSYLVANIA, APRIL 12, 13 AND 14, 1948. Pp. 207. Pittsburgh: Carnegie Press, 1948.

The conference was held to promote the interchange of experience among teachers of medicine, law, divinity, engineering and business. Three sessions were held, relating to: the objectives of professional teaching, 7 papers; the content and the method of professional education, 6 papers; social and humanistic aspects of professional education, 5 papers. The medical member of the planning committee is William M. Beckman, of Harvard University. The book will be of concern to educators in general and to teachers and deans in the disciplines mentioned. To medical teachers the following papers will be of special interest: "The Clinical Training of the Medical Student," by James H. Means, Jackson Professor of Clinical Medicine, Harvard Medical School; "A Social Worker Looks at Medical Education," by Eleanor E. Cockeril, professor of social case work, University of Pittsburgh; "The Physician as a Comprehensive Human Biologist," by John Romano, professor of psychiatry, University of Rochester School of Medicine and Dentistry.

OUTLINE OF HISTOLOGY. By Margaret M. Hoskins, Ph.D., and Gerrit Bevelander, Ph.D., New York University. Second edition. Pp. 113, with 56 illustrations. Price $3.50. St. Louis: C. V. Mosby Company, 1948.

A brief survey of the elementary morphologic aspects of histology is presented in 286 pages, including interleaves for notes, and with one third of the text devoted to dental histology. There are 136 figures, mainly diagrams, including 2 colored plates on blood and several photographs of sections of teeth. There are no references to the literature. Except in the treatment of the nervous system, physiologic aspects are minimized. It is difficult to understand the statement, "In fresh blood, all leucocytes look much alike, and it is only the red cells which can be studied to advantage in the unstained drop of blood." Greater specificity might be preferred in the statement, "The spleen is interposed in the blood stream to remove *impurities* from the blood." (The italics are the reviewer's.) In general, cytologic details are omitted. In some sections the characteristics of the tissues are well summarized.

MEDICAL WRITING: THE TECHNIC AND THE ART. Morris Fishbein, M.D., editor of *The Journal of the American Medical Association*, with the assistance of Jewel F. Whelan, assistant to the editor. Second edition. Pp. 292, with 36 illustrations. Price $4. Philadelphia: The Blakiston Company, 1948.

This guide to medical writing was first published as a small pamphlet in 1910. It is now a unique guide to the preparation of medical manuscripts of all kinds, especially, of course, those intended for any of the publications of the American Medical Association. Its use will help to advance the standards of medical writing.